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RELATION OF TEMPERATURE TO REPRODUCTION IN SUGAR BEETS [1]

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INTRODUCTION

The influence of temperature on sexual reproduction in the sugar beet (*Beta vulgaris* L.) has long been considered a subject of importance. Shaw (*14, p. 112*),[2] strongly influenced by some of the work of Klebs on reproduction in plants, studied the problem extensively and concluded that "the range of temperature within which reproductivity is determined evidently lies between 2.75° C. and 10° C., or very near those limits." Shaw showed that at temperatures below 1.75° the beets remained dormant, and he believed that this dormancy, imposed by low temperature, was responsible for the failure of such beets to develop reproductively. He reasoned that some areas were unfavorable for the reproductive development of beets because silo temperatures were too cool during the winter and because the transition period in the spring, between the prevalence of temperatures too low to allow planting and the incidence of unfavorably high temperatures, was too short to afford an adequate period of "restrained growth." Shaw based his conclusion that 10° is near the upper limit at which reproductivity is determined on a comparison of winter temperature records and his observation of the fact that only part of the plants produced seedstalks in fields of beets planted during November in the Salt River Valley of Arizona.

Shaw pointed out that the mean winter temperature of the natural habitat of the wild beet (*Beta maritima* L.) along the northern coast of the Adriatic Sea insured reproductivity in beets. A mean temperature curve for that area was composited from the mean temperatures of Venice, Trieste, Fiume, Pola, and Zara. By a composite curve of mean temperatures for the coastal area of Oregon and Washington, derived by averaging the mean temperatures of Portland and Roseburg, Oreg., and Seattle, Wash., Shaw showed that the winters of that area are similar to those in the natural habitat of the wild beet. The curves compared by Shaw indicated that the mean temperature range that he considered favorable to "reproductive determination" extended over an even longer period in the coastal area of Oregon and Washington than on the northern coast of the Adriatic.

Pack (*13*) found that the most favorable storage temperature for inducing reproductive development in sugar beets was near 4.4° C., but he did not confirm Shaw's conclusion that storage at temperatures near 0° induced dormancy and thus failed to induce reproductive

[1] Received for publication May 10, 1944.

[2] Italic numbers in parentheses refer to Literature Cited, p. 68.

growth. Pack (*12*) also found that sugar beets which had been stored under optimum temperature conditions (4°) for seed production could be forced into the various degrees of reproductiveness, ranging from true seed types to almost completely vegetative types, by controlling the time of year that mother beets were planted out. Early planting gave the best reproductive development. The fact that in the late plantings some of the beets developed vegetatively suggests that the high temperatures of late spring and early summer caused a reversal of the reproductive process.

Steinberg and Garner (*15*) found that without any cool-temperature exposure a selected strain of sugar beet [3] could be induced to flower at 73° F. by the use of continuous high-intensity illumination. Flowering did not occur at 73° when the plants were exposed to daily illumination periods of 18 hours, but it did occur at 60° and at 65° under 18-hour photoperiods. With continuous illumination, the period from germination to flowering decreased as the temperature was raised, being 65 days at 60°, 48 days at 65°, and 39 days at 73°.

Owen et al. (*11*) showed that in a sugar-beet variety of extremely low bolting tendency storage for 3 months at 33° to 36° F. did not induce bolting except when the plants were transplanted to an environment favorable for reproduction (affording long photoperiods and cool temperatures). A longer period of storage (172 days) of beets of the same variety and at the same temperature resulted in bolting in all the plants, even in an environment unfavorable for bolting (affording short photoperiods and warm temperatures). Evidently a high degree or large amount of thermal induction had been finally effected at the cool temperature by prolonging the storage period.

Apparently either photoperiodic induction or thermal induction, acting independently, can carry the reproductive process far toward completion. The effects of light and temperature on reproduction in the sugar beet seem to be complementary.

The studies reported in this paper deal principally with the relation of temperature to reproductivity in the sugar beet.

MATERIALS AND METHODS

Storage experiments were conducted with eight curly-top-resistant varieties of sugar beets. These varieties, U. S.[4] 15, U. S. 22, S. L.[4] 68, S. L. 234, S. L. 235, S. L. 979, S. L. 1–11, and S. L. 1–121, were known to differ widely in bolting tendency. U. S. 15, S. L. 234, and S. L. 979 require a relatively long cool-temperature treatment before they can be induced to form seedstalks and are, therefore, termed nonbolting varieties. U. S. 22, S. L. 235, S. L. 1–11, and S. L. 1–121 are intermediate in bolting tendency. S. L. 68 requires a relatively short period of cool-temperature exposure to induce bolting and is therefore usually referred to as an easy-bolting variety.

Cool-temperature exposure induces changes that result in subsequent reproductive development when the sugar beet is grown under long daily photoperiods. This process is referred to herein as thermal induction and is measured by the relative rate and percentage of plants bolting. Warm-temperature exposure, following adequate thermal induction, was found to reduce or neutralize the effect of

[3] The seed, supplied by G. H. Coons, Division of Sugar Plant Investigations, was from the easy-bolting variety 00569–0.

[4] Varieties designated "U. S." are commercial varieties grown in the western part of the United States. Those designated "S. L." are varieties bred at the U. S. Sugar Plant Field Laboratory, Salt Lake City, Utah.

cool-temperature exposure and is referred to as reversal of thermal induction.

"Bolting," as used in this paper, refers to the appearance of a seed-stalk, whether or not subsequent flowering occurs (*10, 11, 16*).

To avoid misunderstandings and confusion regarding expressions relating to temperature ranges, the writer proposes to use certain arbitrarily chosen terms with meanings as follows: Low, near or below freezing; cool, above freezing to about 15° C.; warm, about 15° to 30°; and high, above 30°.

Sugar beets were stored at a series of temperatures, beginning at the lowest temperature that could be used without injury to the roots and increasing, by 2° to 3° C., up to the maximum temperature at which thermal induction of reproductive development occurred. Lack of adequate facilities limited the range of the experimental storage temperatures, especially between 10° and 15°. Fairly close control was maintained on temperatures at or slightly below 0°, but temperatures above about 1.5°, in the commercial cold-storage rooms used, were less subject to control. Maximum- and minimum-registering thermometers placed with each lot of beets usually showed a variation of less than 0.7° in lots stored at temperatures lower than 1.5°, whereas the temperature of other lots varied as much as 2°. For this reason the average maximum and minimum temperatures are usually given as the range for each stored lot. In one of the later experiments on reversal of thermal induction some of the sugar beets were stored in electrically heated incubator ovens that were placed in a cool room. This was found to be a more troublesome method of storage owing to the condensation of moisture on the inside of the ovens. The average temperatures recorded in these later experiments are the means of twice-daily readings.

In most experiments the sugar beets were taken from the field in midsummer to avoid the thermal induction of reproductive development that occurs naturally with fall temperatures. A comparison was also made between roots stored at various known temperatures and those that had remained in the field near Harrisburg, Oreg., during most of the winter.[5] The sugar beets that were used in experiments to determine the reversibility of thermal induction were taken from overwintering fields after sufficient cool-temperature exposure to cause most plants to bolt when subsequently replanted in a favorable photoperiodic environment.

All lots of sugar beets were taken from moist soil, and the leaves were carefully trimmed off without injury to the central crown bud. They were then placed in large, clean, galvanized-iron cans and taken to storage. Drying of roots during storage was avoided by keeping a small amount of water in the cans. False bottoms of perforated metal, 1 to 2 inches high, were placed in each can to allow air circulation within the can and to prevent the beets from coming in direct contact with water. At storage temperatures above 15° C., wet excelsior, placed both above and below the roots, was used in some instances, and such lots were removed frequently for examination and to aerate the cans.

Roots held at low temperatures were allowed to warm up slowly when taken from storage. The leaf growth on beets stored at warmer

[5] Sugar beets received from Harrisburg, Oreg., were shipped to the writer through the courtesy of the West Coast Beet Seed Co.

temperatures was trimmed off, and the roots were replanted in a greenhouse floor bed or in the field. Greenhouse temperatures were kept relatively warm, and field plantings were delayed until late spring to avoid further thermal induction after replanting.

Since the effects of long daily photoperiods and prolonged cool-temperature exposure are apparently complementary in inducing reproductive development in biennial beets, long photoperiods (either 17 to 18 or 24 hours daily) were chosen so as to bring out the differential bolting response to thermal treatments. During tests made in the greenhouse in the winter and early spring, artificial illumination (25 to 50 foot-candles) supplementary to daylight was supplied by Mazda lamps equipped with reflectors.

EXPERIMENTAL RESULTS

THERMAL INDUCTION IN SUGAR BEETS TAKEN FROM THE FIELD DURING THE SUMMER

Six lots of sugar beets of the easy-bolting variety S. L. 68 were stored at different temperatures from July 22 to September 13, 1939. All lots were replanted in a warm greenhouse September 13, and were

TABLE 1.—*Effect of various storage temperatures on thermal induction in sugar beets as shown by bolting response of different varieties replanted in a warm greenhouse under continuous illumination or 17- to 18-hour photoperiods*

Variety, storage period, photoperiod, and treatment designation	Storage temperature		Beets observed	Beets bolting at end of indicated period (days) after being replanted											
	Average minimum	Average maximum		17	24	25	28	31	34	40	45	47	50	53	56
S. L. 68 (July 22–Sept. 13, 1939); continuous illumination:	°C.	°C.	No.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.
A	−0.8	0.7	30	0	0			3.3			56.7				
B	1.8	2.7	30	0	3.3			3.3			63.3				
C	1.3	3.2	30	0	3.3			10.0			70.0				
D	2.7	3.4	30	0	16.7			53.3			90.0				
E	6.2	7.6	30	23.3	80.0			100.0			100.0				
F	9.5	10.2	30	38.0	82.9			93.1			100.0				
S. L. 68[1] (Sept. 6–Oct. 25, 1940); 17- to 18-hour photoperiods:															
A	−1.0	−.5	50		0			0		0				10.0	
B	0	1.7	50		0			0		10.0				26.0	
C	4.0	6.9	50		18.0			52.0		90.0				100.0	
D	5.5	6.7	50		34.0			72.0		92.0				100.0	
U. S. 15 (July 22–Oct. 18, 1939[2]); continuous illumination:															
A	−1.1	.4	30				0		0	6.7			10.0		
B	.6	2.1	30				0		0	3.3			13.3		
C	1.4	2.3	30				0		0	0			20.0		
D	2.4	3.1	30				0		3.3	10.0			16.7		
E	4.7	6.3	30				30.0		60.0	70.0			86.7		
F	8.4	9.7	30				23.3		33.3	43.4			53.3		
U. S. 22[3] (Nov. 27, 1940–Jan. 16, 1941); 17- to 18-hour photoperiods:															
A	−1.3	−.8	35			0						5.7			5.7
B	.8	2.0	40			0						17.5			20.0
C	4.2	5.3	40			7.5						67.5			80.0
D	4.4	5.8	40			22.5						72.5			87.5

[1] The beets were taken from the field Aug. 28 and held at 0.6° to 1.7° C. until Sept. 6, 1940, when they were divided into 4 lots and stored at different temperatures.

[2] Storage temperatures were the same as those for S. L. 68 for first 53 days; from Sept. 14 to Oct. 18, lower outside temperatures affected storage temperatures enough to lower nearly all the general averages.

[3] The beets were taken from the field Aug. 28 and held at approximately 0.5° to 1.0° C. for 1 month, then at −1.0° to −0.5° until Nov. 27, 1940, when they were divided into 4 lots and stored at different temperatures.

continuously illuminated thereafter. Data in table 1 and figure 1 indicate that thermal induction proceeded at a relatively slow rate at −0.8° to 0.7° C., and that the maximum rate was at temperatures above 6° and probably near 10°. The data also indicate that even at the lowest temperature, where the rate of thermal induction was

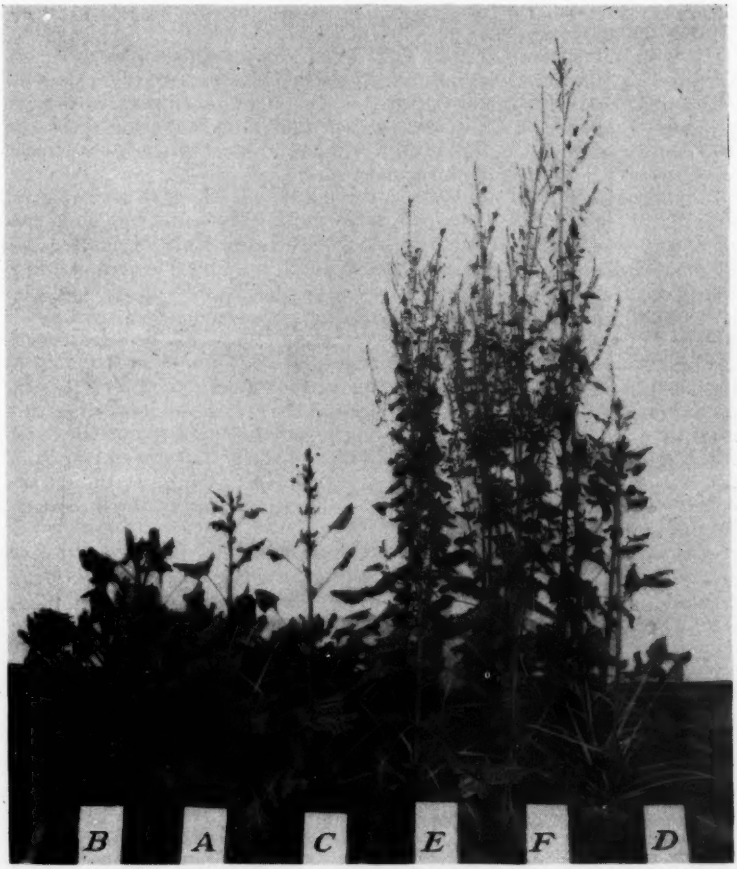

FIGURE 1.—Effect of different storage temperatures on thermal induction in sugar beets as shown by the bolting response of variety S. L. 68 replanted in a warm greenhouse under continuous illumination after 53 days of storage. Average minimum and maximum storage temperatures of respective treatments: *A*, −0.8° to 0.7° C.; *B*, 1.8° to 2.7°; *C*, 1.3° to 3.2°; *D*, 2.7° to 3.4°; *E*, 6.2° to 7.6°; *F*, 9.5° to 10.2°. Photographed November 8, 1939, 56 days after being replanted.

relatively slow, the process went far enough to result, after only 53 days of storage, in a large percentage of plants bolting.

In another test with variety S. L. 68, the roots were taken from the field August 28, 1940, and held at 0.6° to 1.7° C. until September 6. On the latter date they were divided into four lots and stored at different temperatures until October 25, when they were replanted in

the greenhouse under 17- to 18-hour photoperiods. The data in table 1 and figure 2 show that there was very little thermal induction at −1.0° to −0.5° and that there was an increase in the rate of the process at each increase in storage temperature. As in the previous experiment with this variety, a relatively short storage period, even at the lowest temperature, afforded sufficient thermal induction to result in bolting in some plants.

The photographs in figure 2 show even greater differences in reproductive growth than are indicated by the data in table 1. Most of the sugar beets previously stored at the higher temperatures were flowering, whereas only a few seedstalks had been initiated in the beets stored at temperatures below 0° C., and most of these seedstalks were semivegetative in appearance.

Sugar beets of the nonbolting variety U. S. 15 were stored from July 22 to October 18, 1939, at approximately the same range of temperatures used that season with variety S. L. 68 (table 1). Because of lower outside temperatures, the average minimum and maximum temperatures in this test (table 1) were nearly all cooler than the corresponding temperatures in the former experiment.

Decayed spots developed in the crowns of some of the sugar beets of variety U. S. 15 stored at the two highest temperatures (table 1, treatments E and F), necessitating rather severe trimming of the crowns before the beets were replanted. It was thought that this might reduce bolting, but it is now believed that such is not the case. Although the tallest seedstalks were among those that developed from the lot stored at 8.4° to 9.7° C. (treatment F), the most rapid rate and the highest percentage of plants bolting occurred in the lot stored at 4.7° to 6.3° (treatment E).

The rate of thermal induction in U. S. 15, as in S. L. 68, was slower at the lower storage temperatures, as indicated by the slower bolting rate and smaller percentage of plants bolting. Unlike S. L. 68, however, U. S. 15 did not receive enough thermal induction for complete bolting at the storage temperature previously found to be the most favorable, even though the storage period was considerably longer than in the tests with S. L. 68. Obviously, U. S. 15 requires more extensive thermal induction. Another point believed to be significant is that apparently a storage temperature of 8.4° to 9.7° C. was higher than optimum for thermal induction in U. S. 15. Perhaps a much longer storage period at a relatively cool temperature would be necessary for adequate thermal induction in this variety.

A storage experiment with variety U. S. 22 was started August 28, 1940. The roots were taken from the field and held for 31 days at approximately 0.5° to 1.0° C.; then they were moved to a storage chamber in which the temperature ranged from −1.0° to −0.5°. On November 27, the beets were divided into four lots, which were stored respectively, at the temperatures shown in table 1. The temperature in the coldest chamber was reduced to about −2.0° for the first 2 weeks, when it was observed that nearly all the roots were injured superficially by freezing. However, after the beets were transplanted, only one of the plants failed to grow, and four others died during the experiment. It seems probable that the very limited amount of thermal induction in this case occurred during the 31 days of preliminary storage, when the temperature was at all times slightly above

0°, and that during the 110 days when the temperature was continuously below 0° the sugar beets were so dormant that thermal in-

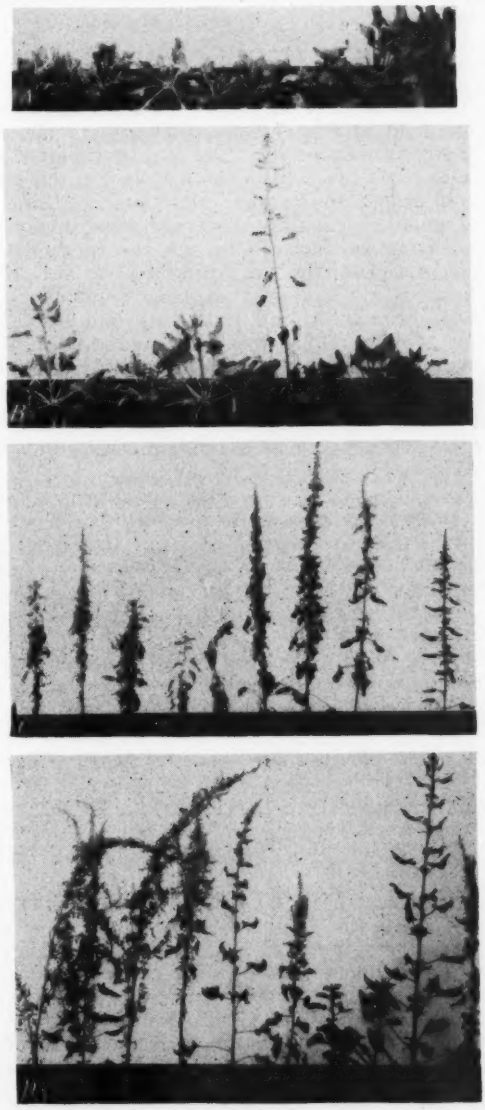

FIGURE 2.—Effect of different storage temperatures on thermal induction in sugar beets as shown by the bolting response of variety S. L. 68, replanted in a warm room under 17- to 18-hour photoperiods after 49 days of storage. Average minimum and maximum storage temperatures of respective treatments: *A*, −1.0° to −0.5° C.; *B*, 0° to 1.7°; *C*, 4.0° to 6.9°; *D*, 5.5° to 6.7°. Photographed January 15, 1941, 82 days after being replanted.

duction was practically arrested. However, when beets that had been kept virtually dormant at near 0° were stored at approximately 5° for 50 days, a high percentage of bolting resulted, indicating that thermal

induction at the higher temperature was fairly rapid. The data also indicate that at similar temperatures U. S. 22 requires a longer storage period than does S. L. 68 to induce bolting in an equal percentage of plants.

THERMAL INDUCTION IN SUGAR BEETS TAKEN FROM THE FIELD DURING THE WINTER

Sugar beets taken from the field in the summer and those taken from the field in winter were compared to determine the effect of cool-temperature storage. The results reported in table 1 were obtained with roots taken from the field before the occurrence of temperatures cool enough for thermal induction. In these tests, therefore, only the thermal effects during storage were involved. In contrast to tests with summer-harvested roots, an experiment was conducted in which sugar beets of the nonbolting variety S. L. 979 that had grown in the field at St. George, Utah, from September 16 to December 6, 1939, were brought to Salt Lake City and stored at various temperatures for 44 days. They were then replanted in the greenhouse under continuous illumination. One lot of plants of the same variety, which had grown in the field at Salt Lake City from August 16, 1939, to January 19, 1940, was transplanted to the greenhouse with the stored lots. The treatments used and results obtained are given in table 2.

TABLE 2.—*Effect of different storage temperatures on thermal induction in sugar beets as shown by bolting response of variety S. L. 979 replanted in a warm greenhouse under continuous illumination*

[Storage period 44 days, Dec. 6, 1939, to Jan. 19, 1940]

Treatment [1]	Storage temperature		Beets [2] bolting at end of indicated period (days) after being replanted		
	Average minimum	Average maximum	26	36	52
	° *C.*	° *C.*	*Percent*	*Percent*	*Percent*
A	0.2	1.3	9.3	37.2	51.2
B	1.6	2.3	11.6	44.2	65.2
C	4.7	6.7	32.6	55.8	69.7
D	6.1	7.1	32.6	58.2	74.5
E	6.5	8.2	34.9	62.8	72.1
F			67.5	90.7	95.4

[1] Beets receiving treatments A to E were grown at St. George, Utah; those receiving treatment F were grown near Salt Lake City, Utah, and transplanted directly from the field.
[2] Percentages are based on a total of 43 beets observed for each treatment.

The sugar beets taken from the field in early December and stored at 0.2° to 1.3° C. for 44 days bolted at a faster rate and in higher percentages than did U. S. 15 beets of comparable inherent bolting tendency taken from the field in July and stored for 88 days at approximately the same temperature (treatment A in table 1). The sugar beets that were transplanted directly from the field at Salt Lake City to the greenhouse in January bolted even more rapidly and in greater numbers than the sugar beets from St. George that had been stored. Obviously, some thermal induction of reproductive development had occurred in both lots during the time they were exposed to fall and early-winter temperatures in the field. On the other hand, as shown by previous experiments, in the beets taken from the field in July, bolting was thermally induced only during storage.

Another comparison of the effect of thermal exposure of sugar beets in the field with that of thermal exposure during storage was made in a field test planting in 1940. Mother beets of the nonbolting variety U. S. 15 were harvested near Salt Lake City during October 1939 and stored at two different temperatures. Steckling beets of the same variety were taken from an overwintering beet-seed field near Harrisburg, Oreg., March 10, 1940. The three lots were replanted in rich garden soil at Heber City, Utah, April 5, 1940. Photographs taken 77 days after the beets were replanted (fig. 3) show that the sugar beets that had overwintered in the field (fig. 3, *C*) were beginning to flower before seedstalks were produced on the lot stored at 0° to 1.7° C. (fig. 3, *A*). The beets stored at an average temperature of 4.4° (fig. 3, *B*) were intermediate in development between the two extremes. The more advanced stage of reproductive development of the steckling beets indicates that thermal induction may be more completely accomplished by overwintering sugar beets under optimum conditions in the field than by storing them at favorable temperatures.

REVERSIBILITY OF THERMAL INDUCTION

There has been some difference of opinion regarding the reversibility of developmental processes in plants. According to the Imperial Bureau of Plant Genetics (*5*), Lysenko believed that these processes take place in definite steps and sequence, that a qualitative change in the protoplasm induces morphological changes, and that the steps that must take place in the plant before it reaches sexual maturity are irreversible. Ljubimenko and other Russian workers (see *5*) believed that the presence of a developmental hormone is responsible for the morphological changes involved in reproductive development and that the developmental processes are reversible. Many instances have been reported showing that the type of development may be reversed. Chroboczek (*3*) reversed the development of red garden beets several times in the same plant by changing the thermal and photoperiodic environment. This method was also used by Owen (*9*) to produce semivegetative seedstalks in sugar beets for use as cuttings.

Since thermal induction in sugar beets occurs only at relatively cool temperatures, it seems probable that substances conducive to bolting are formed in beets stored at those temperatures and that at warmer temperatures these substances are not formed or at least not accumulated. The rate of thermal induction, within the favorable temperature range, increases as the temperature rises. It is probable, therefore, that biochemical processes that are accelerated by an increase in temperature are involved. In view of this, if relatively warm storage temperatures were found to result in reversal of thermal induction, it is reasonable to expect that the rate of reversal would be relatively rapid. Any observed reversal could be attributed to thermal alteration of the biochemical equilibrium of the plant, resulting in a change in the direction of the biochemical processes involved. This line of thought led to experiments in which sugar beets that had received some thermal induction were stored at an intermediate range of temperatures (9° to 25° C.) for short periods to determine whether reversal could be so induced.

The sugar beets used in one such test were of the variety S. L. 1–121, classed as intermediate in bolting tendency. They were

taken from an overwintering seed field near St. George, Utah, about January 20, 1942. One lot was directly replanted under continuous

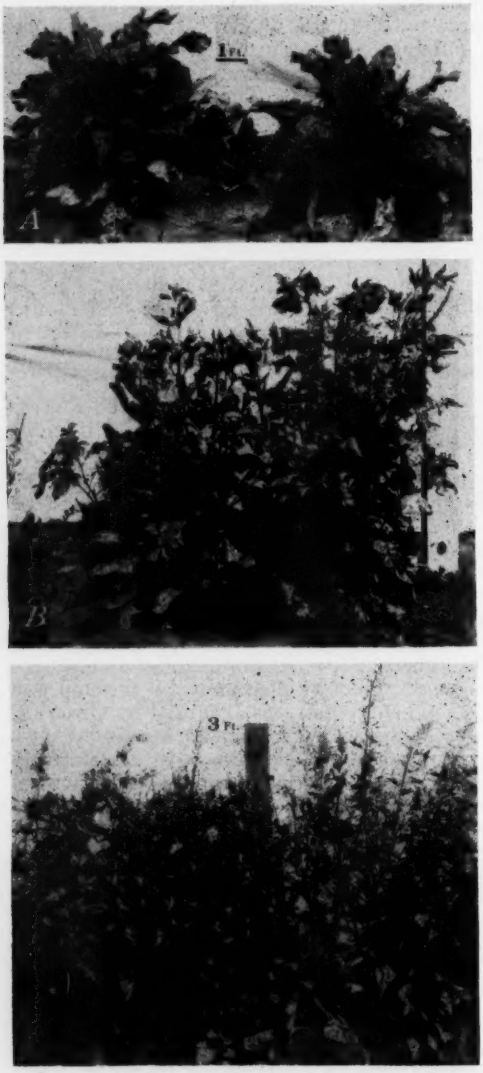

FIGURE 3.—Comparison of effects of different storage temperatures and of overwintering in the field on thermal induction in sugar beets as shown by the bolting response of variety U. S. 15, replanted in the field at Heber City, Utah, April 5, 1940: *A*, Mother beets stored at 0° to 1.7° C.; *B*, mother beets stored at an average of 4.4°; *C*, steckling beets taken from an overwintering beet-seed field near Harrisburg, Oreg., March 10, 1940. Photographed June 21, 1940.

illumination in a warm greenhouse room; another lot was stored at 17° to 18° C., and a third lot was stored at 23° to 24°. Plantings from each stored lot were made at semiweekly intervals.

The data in table 3 show that there was some reduction in the rate of bolting of plants that were stored at 17° to 18° C. for a period of about 10 days. All the beets stored at this temperature, however, had bolted 51 days after being replanted. There was a much greater reduction of bolting in beets that were stored at 23° to 24°. Of the beets stored at this temperature for 21 days, only 20 percent bolted in 51 days.

TABLE 3.—*Effect of storage at warm temperatures on the reversal of thermal induction in sugar beets as shown by the bolting response of variety S. L. 1–121 when replanted in a warm greenhouse under continuous illumination*

Storage temperature and period	Beets observed	Beets bolting at end of indicated period (days) after being replanted				
		23	30	37	44	51
	Number	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Check (not stored)	20	30	90	100	100	100
17° to 18° C.:						
3 days	20	20	75	95	100	100
7 days	20	5	75	95	100	100
10 days	10	0	40	100	100	100
13 days	10	10	80	90	90	100
23° to 24° C.:						
3 days	20	0	30	90	100	100
7 days	20	0	50	85	90	90
10 days	20	0	55	75	75	75
13 days	20	0	40	60	80	85
17 days	20	0	5	35	45	50
21 days	20	0	5	15	20	20

Sugar beets of the intermediate-bolting variety S. L. 1–11 were obtained from overwintering fields in St. George, Utah, and Talent, Oreg., about January 20, 1942. They were stored at −0.5° C. until April 20, when a sample from each lot was moved to room temperature (average 25.6°) for a period of 16 days before all lots were replanted in the field May 6, 1942. Of the beets given the warm-temperature treatment only 1 percent bolted, whereas 92.6 percent of the others bolted (table 4 and fig. 4).

TABLE 4.—*Reversal of thermal induction in sugar-beet variety S. L. 1–11 by a short period of storage at a relatively warm temperature*

Treatment	Average storage temperature		Beets observed	Beets bolting	
	Jan. 20 to Apr. 20	Apr. 20 to May 6		June 16	July 22
	° *C.*	° *C.*	*Number*	*Percent*	*Percent*
A	−0.5	−0.5	1,699	87.3	92.6
B	−.5	25.6	395	0	1.6

To test further the lower limits of temperatures that result in reversal of thermal induction and to obtain information regarding the rate at which these changes are effected, another test was made in 1943. On March 4, plants of three sugar-beet varieties—U. S. 22 and S. L. 235, which are intermediate in bolting tendency, and S. L. 234, a nonbolting variety—were taken from an overwintering seed field planted in St. George, Utah, September 7, 1942. The beets

of each variety were divided into nine lots. All were stored uniformly at a low temperature until March 24, when five lots of each variety were moved to warmer temperatures. Details of these storage

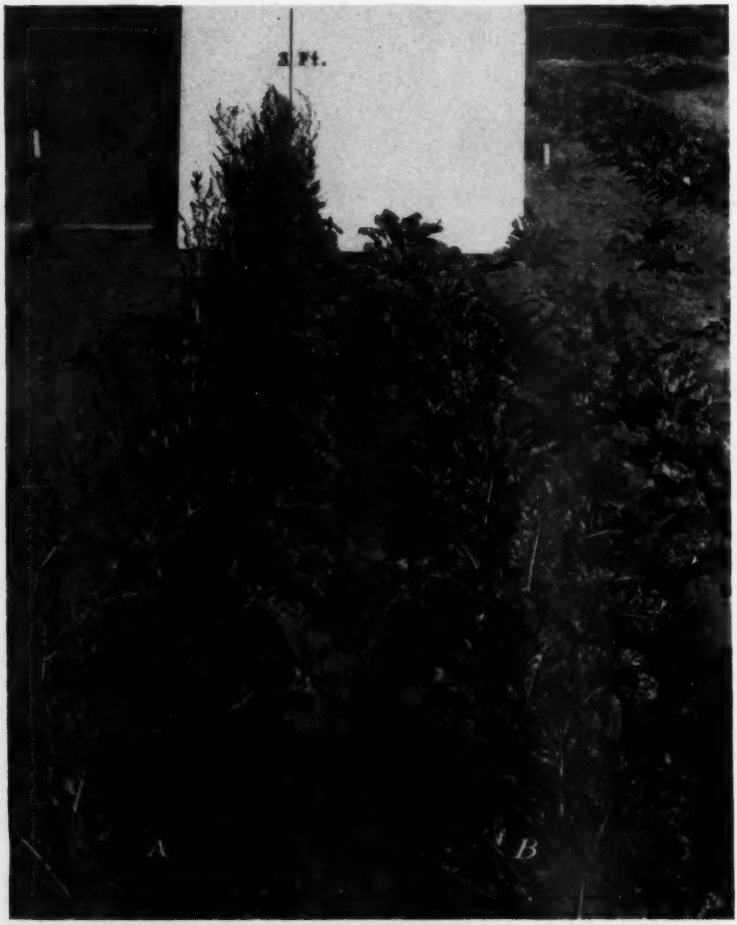

FIGURE 4.—Reversal of thermal induction at a relatively warm temperature. The sugar beets in rows *A* and *B* were taken from an overwintering seed field in January 1942 and stored at −0.5° C. until April 20, when those in row *B* were removed from the low-temperature chamber and stored at approximately 26°. Sixteen days later both lots were replanted in the field. Photographed June 28, 1942.

temperatures are shown in table 5. The other four lots were held at −0.85° C. until April 12, when two more lots from each variety were moved to warm temperatures. The two remaining lots of each variety were kept at −0.85° as checks. All lots of beets were replanted in the field April 29, 1943. Those beets that were seriously injured

or severely affected by curly top were eliminated before bolting counts were made.

Some difficulty was encountered in storing the sugar beets at 16.4° C. for 36 days. Electrically heated incubator ovens placed in a cool room were used as temperature chambers. The beets were placed on trays and water was kept in pans inside the ovens to maintain high humidity. The thermal differential between the inside of the ovens and the outside air caused moisture to condense on the inside and drip on some of the beets. This difficulty resulted in considerable spoilage in these lots. Although the percentage of plants bolting in this lot is based on a small number, the results are in line with the data based on larger numbers in lots in which there was little injury from spoilage.

TABLE 5.—*Effect of storage periods, differing in temperature and duration, on reversal of thermal induction in sugar beets as shown by bolting response of varieties U. S. 22, S. L. 234, and S. L. 235, when replanted in the field April 29, 1943*

Variety	Storage period		Beets observed	Beets bolting at end of indicated period (days) after being replanted		
	Temperature	Duration		30	43	50
	° C.	*Days*	*Number*	*Percent*	*Percent*	*Percent*
U. S. 22	−0.85	36	114	96.5	100.0	100.0
	9.1	36	56	94.6	98.2	100.0
	10.6	36	45	93.3	100.0	100.0
	13.2	36	25	12.0	48.0	56.0
	16.4	36	4	0	0	0
	19.3	17	51	19.6	49.1	51.0
	23.8	17	49	0	4.1	8.2
	[1]	36	34	14.7	20.6	20.6
S. L. 234	−.85	36	83	84.4	96.3	98.8
	9.1	36	51	74.5	98.0	98.0
	10.6	36	39	41.1	79.5	79.5
	13.2	36	17	0	23.5	35.3
	16.4	36	4	0	0	0
	19.3	17	16	6.2	12.5	12.5
	23.8	17	23	0	0	0
	[1]	36	34	0	14.7	20.6
S. L. 235	−.85	36	99	87.9	100.0	100.0
	9.1	36	49	95.9	100.0	100.0
	10.6	36	35	82.9	91.5	97.2
	13.2	36	13	53.8	53.8	69.2
	16.4	36	8	0	0	0
	19.3	17	45	31.1	60.0	62.2
	23.8	17	47	0	2.1	6.4
	[1]	36	29	10.3	24.1	27.6

[1] Beets were stored at average alternating temperatures of 9.1° C. for 16 hours and approximately 26° for hours daily for 36 days before they were replanted.

The data in table 5 show that some reversal of thermal induction occurred at temperatures near 10° to 12° C. About the same amount of reversal was effected in sugar beets stored at 13.2° for 36 days as in similar beets stored at 19.3° for only 17 days. This indicates that the rate of reversal at 19.3° was approximately twice as great as at 13.2°. There was a rather striking reduction in the percentage of beets bolting in the nonbolting variety S. L. 234 stored at 10.6°. In the two other varieties the reduction in plants bolting after storage at this temperature was so small as to be of questionable significance. At 13.2° all the varieties showed pronounced reduction in percentage of plants bolting.

Those sugar beets stored at alternating temperatures for the entire period of 36 days showed a marked reduction in bolting response.

There was evidently greater reversal of thermal induction in the beets stored at these daily alternating temperatures for 36 days than in those stored continuously at 19.3° C. for 17 days, but less than in those

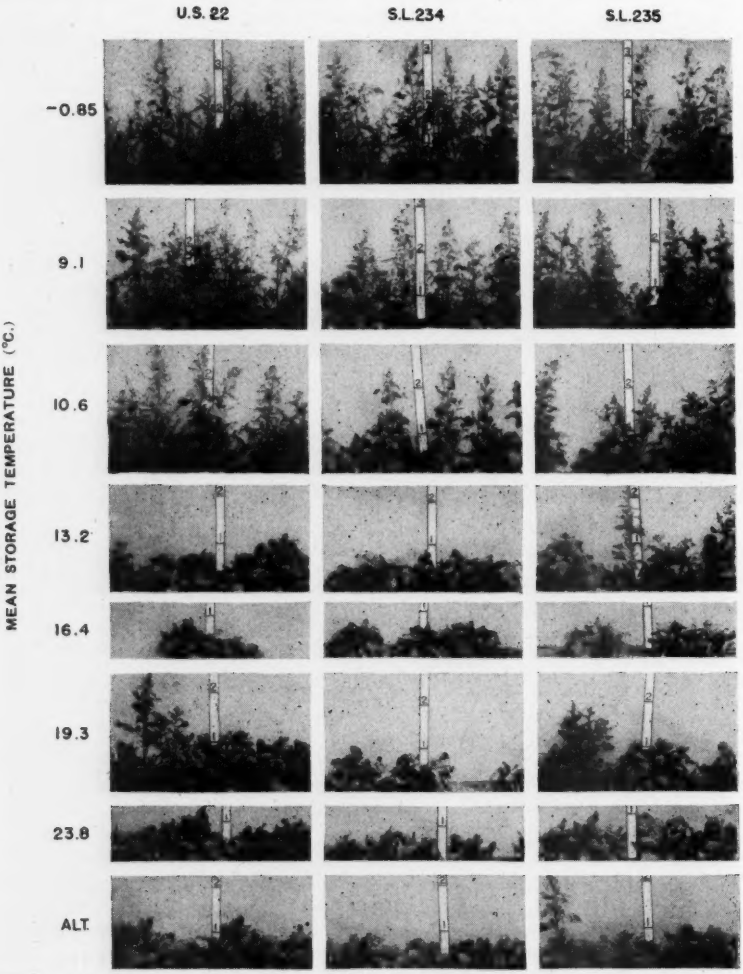

FIGURE 5.—Representative beets from treatments listed in table 5. Storage periods as follows: −0.85° C., for 36 days; 9.1°, for 36 days; 10.6°, for 36 days; 13.2°, for 36 days; 16.4°, for 36 days; 19.3°, for 17 days; 23.8°, for 17 days; daily alternating (Alt.) temperatures, for 36 days. Photographed June 22, 1943, 54 days after being replanted. (Small numbers in each photograph indicate height in feet.)

stored continuously at 23.8° for 17 days. A composite photograph of representative sugar beets in each plot, taken 54 days after being replanted, is shown in figure 5.

DISCUSSION

The existence of a substance or substances that may be responsible for reproductive development in higher plants has been suggested by many writers (*2*, *6*, *7*, *8*). The principal basis for this assumption has been the demonstration that photoperiodically produced substances or stimuli (*1*, *4*) may be transferred from one part of a plant to another through grafts or by manipulating light exposure or foliar growth. Sugar beets apparently develop reproductively as a result of the production and accumulation of a substance or substances in sufficient quantities to cause the morphological changes that occur. The influence of temperature on the rate and reversibility of the process seems to support the assumption that it is of a chemical nature. The data in table 5 show that the rate of reversal was approximately twice as fast at 19.3° C. as at 13.2°. This indicates a temperature coefficient of approximately 3.3 for the rate of reversal between 13.2° and 19.3°. The fact that the temperature coefficient of reversal is high, while not considered as proving the chemical nature of the process, is thought to afford evidence supporting this viewpoint, inasmuch as the usually accepted value of the temperature coefficient of purely physical processes is relatively low, ranging from 1.2 to 1.4 at the temperatures that ordinarily prevail in the plant.

The available data on thermal induction and on the reversal of the process in sugar beets seem to justify the assumption that this induction process may be considered to exist in a state of complete reversibility until sufficient seedstalk development occurs to partially prevent reversal of reproductive development. That this morphological differentiation exerts a restraining influence on reversal is shown by the fact that reproductive development usually continues in spite of the high temperatures that prevail in some seed-growing areas during the period of rapid seedstalk growth and flowering.

A gradient in the amount of thermal induction between the small amount occurring at a relatively warm temperature and the large amount attainable at a cool temperature is indicated by several lines of evidence. At warmer temperatures a little thermal induction will occur if the beets involved have not been thermally induced previously. If the beets involved have already received extensive thermal induction, holding them at the warmer temperature will result in reversal of thermal induction toward the equilibrium level for that temperature.

An example of one line of evidence of the gradient in extent or amount of thermal induction has been reported previously (*11*). Clone No. 138 is a nonbolting type and remains vegetative under conditions which cause most sugar beets to develop seedstalks. An 87-day treatment of plants of this clone at 0.5° to 2.2° C. did not induce bolting except when followed by a favorable environment for bolting, that is, one affording a long photoperiod and moderately cool temperature. However, storage for 172 days at 0.5° to 2.2° induced bolting even in an environment that was unfavorable, that is, one affording short photoperiods and relatively warm temperatures. This indicates that there was more extensive thermal induction as a result of the longer period of storage at cool temperatures. Experiments with other varieties gave similar results.

Another line of evidence is afforded by comparing the responses of the varieties S. L. 68 and U. S. 15. The former variety is an easy-

bolting type, whereas the latter is decidedly nonbolting. Plants of S. L. 68, stored 53 days at 9.5° to 10.2° C., received enough thermal induction to effect complete bolting under very favorable photoperiodic conditions. U. S. 15, on the other hand, bolted incompletely under a similar photoperiodic environment after storage for 88 days, even at 4.7° to 6.3°, a temperature evidently more favorable for thermal induction (table 1) than the higher temperature found to be adequate with S. L. 68. There was more bolting in beets of variety U. S. 15 stored at 4.7° to 6.3° than in those stored at 8.4° to 9.7°. From these facts it appears that the temperature limitation in the relatively warmer ranges permits an amount of thermal induction that is adequate for complete bolting in S. L. 68, but inadequate in the case of U. S. 15.

The bolting responses of the nonbolting variety S. L. 234 and the two intermediate-bolting varieties U. S. 22 and S. L. 235 afford further pertinent evidence. The data in table 5 and figure 5 show that there was an appreciable reduction in bolting of S. L. 234 after storage at 10.6° C. for 36 days; U. S. 22 and S. L. 235 showed little reduction in bolting after storage at 10.6°, but a definite reduction in bolting after storage at 13.2°.

The bolting response of a hypothetical population of beets representing all possible degrees of bolting tendency from easy-bolting to the most nonbolting types and grown at temperatures ranging from 3° to 18° C. for a long period before they are subjected to more rapid growing temperatures and long photoperiods, might be represented by a curve similar to *EF* in figure 6. That is, only the easy-bolting types would bolt after exposure at 12° to 15°; others, of intermediate-bolting tendency, would bolt after exposure at 8° to 10°; and still more nonbolting types might bolt after exposure to cooler temperatures, depending upon the amount or degree of induction necessary to satisfy the thermal-induction requirement of each type. The above-mentioned data seem to indicate that essentially the same result might be approached, either by thermal induction of beets not thermally induced or by reversal of thermally induced ones.

The foregoing facts and inferences seem to support the hypothesis that a high degree of thermal induction is attainable at a comparatively cool temperature, that a low degree is possible at the other extreme of the temperature range within which the process can take place, and that for each degree of temperature between these points there is a corresponding, intermediate degree of thermal induction attainable. According to this view there is a gradient in the degree of thermal induction, dependent on temperature.

The direction of the thermally induced changes (thermal induction or reversal) is thus dependent upon the storage temperature and the amount or degree of thermal induction attained before storage. Thermal induction seems always to increase, although slowly, at temperatures near the freezing point. The amount of thermal induction attainable as temperatures are increased, decreases until, at 12° to 15° C., little or none occurs. At temperatures above about 15° any appreciable amount of thermal induction effected previously is reversed. If a large amount of thermal induction has been effected before experimental storage, a partial reversal will occur if the storage temperature, even though cool, is higher than that to which the beets were previously exposed. On the other hand, if no thermal

induction has been effected previously, an observable amount of thermal induction may be effected in an easy-bolting variety at temperatures even as high as 12° to 15°. Thus there is an intermediate range of temperatures at which either thermal induction or reversal may occur. This is believed to indicate a shift in the biochemical equilibrium of the plant as a result of change in temperature. Since the process is thermally reversible, it might be represented by an equation in which *Ro* represents a condition favoring reproductive development while *rO* represents a condition favoring vegetative development ($Ro \rightleftarrows rO$). A graphic illustration of this concept is shown by the curve *EF*, drawn in relation to the left and base axes of figure 6. It should be borne in mind that the curve illustrates the ultimate amount or degree of thermal induction that might result at a given temperature regardless of the direction of approach or the time required to establish equilibrium.

Temperature also influences the *rate* of thermal induction and of reversal. The fact that the rate of thermal induction is greatly reduced as storage temperatures approach the freezing point indicates that this process is retarded as the biochemical processes involved approach quiescence. Within the favorable temperature range for thermal induction, the rate increases as the temperature rises. The fact that the rate of reversal similarly increases as the temperature rises indicates that, even though the directions of these changes in developmental response are diametrically opposite, there is continuity in the rate of the changes induced by temperature. In other words, the relation of temperature to the *direction* or *kind* of change is discontinuous, as proved by the reversible nature of the process, but the relation of temperature to the *rate* at which these changes occur is continuous. The curves *GH* and *IJ*, drawn in relation to the base and right axes of figure 6, illustrate the writer's concept of the relation between temperature and rate of thermal induction and reversal, respectively.

The interaction of the two previously described relations (illustrated by the curves *EF* and *GH*—*IJ* in fig. 6) represents the writer's interpretation of the reproductive response of sugar beets to storage temperatures when such thermal exposure is followed by a favorable photoperiodic environment. It is evident that, although a large amount or high degree of thermal induction may be attained by storing sugar beets at a temperature near freezing, a long period of time is required because of the reduced rate of change that occurs at such temperatures. At higher temperatures, e. g., 6° to 8° C., the rate of induction is more rapid and the amount or degree of induction possible at such temperatures is probably adequate to induce bolting in most varieties, provided the beets are replanted in an environment affording temperatures cool enough and photoperiods long enough to prevent reversal from occurring before reproductive development is initiated. Reversal of thermal induction in plants after they are replanted in warm weather is thought to indicate another reason why Pack (*12*) found late planting of mother beets unfavorable for seed production.

Pack's (*13*) observation of complete bolting of beets after storage at 0° C. was probably due to the fact that there may have been some thermal induction in the field in the fall before harvest. Pack's dates of storage in one test are not stated, but the beets used in another

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test were apparently received from Fort Collins, Colo., and experimental storage started during the latter part of November. The beets were replanted in the field at Salt Lake City, Utah, on April 1, after about 120 to 130 days of storage. Fall temperatures at Fort Collins and spring temperatures at Salt Lake City are regularly cool enough to result in considerable thermal induction before November and also after April 1. Pack, therefore, was measuring the thermal

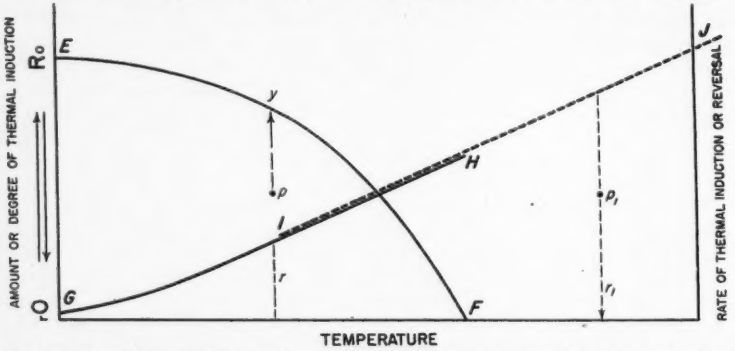

FIGURE 6.—Diagram illustrating a hypothesis concerning the separate effect of temperature on the amount or degree of thermal induction attainable and also its effect on the rate at which these thermally induced changes occur. Since the two relations have a common axis (temperature) they are illustrated in the same figure. The curve *EF*, drawn with respect to the left and base axes of the figure, indicates the relation of temperature to the amount or degree of thermal induction possible at a given temperature. Thermal induction can occur only under conditions existing within the boundary of this curve. (At temperatures higher than *F*, only substances favorable to vegetative development (*rO*) are accumulated.) The curve *GH*, drawn with respect to the base and right axes of the figure, indicates the initial rate of thermal induction at temperatures where thermal induction is possible. The curve *IJ*, which is an extension of the curve *GH*, represents the influence of temperature on the initial rate of reversal at temperatures where reversal occurs. The direction (thermal induction or reversal) of the changes that occur at temperatures illustrated by the overlapping portion of the curves *GH* and *IJ* depends upon the amount or degree of thermal induction attained before experimental storage. If beets that have previously been thermally induced to an amount or degree represented by points *p* or p_1 are stored at temperature *p*, thermal induction will occur at rate *r* until an amount or degree of induction represented by point *y* on the curve *EF* is approached. If a similar beet is stored at temperature p_1, reversal will occur at rate r_1 until complete reversal is effected.

induction effected both before harvest and after replanting as well as during experimental storage.

Information regarding the effects of temperature on reproduction of sugar beets as it applies to overwintering the plants in the field is of great interest in the United States, because this has become the predominant American method of sugar-beet seed growing. The fact that prevailing temperatures in some of the seed-growing areas are too warm (*10*) has emphasized the desirability of cooler temperatures, but Shaw's (*14*) results and those obtained by the writer have shown that, for optimum thermal induction, temperatures should not be too cool. From the standpoint of temperature, the most desirable seed-growing area, especially for the reproduction of nonbolting varieties, would be one in which moderately cool temperatures prevail continuously over a long period or one in which the transition

from warm to low temperatures in the fall and from low to warm temperatures in the spring is long and gradual.

The rapid rate at which reversal of thermal induction occurs at warm temperatures (tables 4 and 5 and figs. 4 and 5) suggests an explanation for the failure of nonbolting varieties to reproduce satisfactorily in some of the warmer seed-growing areas. Warm midday temperatures during even the cooler periods may so counteract the effect of favorably cool night temperatures that little net thermal induction results. Then, too, short periods of warm weather occur in these areas during late fall, winter, and early spring. Such unfavorable conditions, occurring before initiation and early development of seedstalks have been effected, may be very detrimental to seedstalk development. Tolman (*16*) found differences as great as 10° C. between the crown temperatures of normal and small-sized or defoliated beets during midday in February, and he showed that these temperatures were correlated with the percentage of plants that remained vegetative or produced no seed. Tolman also showed that certain cultural practices maintained a more favorable thermal environment, which resulted in an increase of plants producing seed, thereby increasing seed yields.

The fact that thermal induction is greatly retarded at temperatures near 0° C. can be utilized in breeding nonbolting varieties of sugar beets. Seed can be planted in the field in the fall; and then in late winter, after thermal induction has been completed only in the easy-bolting types, the beets can be placed in storage at −0.5° to 1.0°. Storage at such temperatures will virtually arrest further thermal induction during the remaining cool months until the beets can be replanted in the field the following spring. The easy-bolting types will then produce seedstalks and can be eliminated. This method has been used with success during two seasons by F. V. Owen [6] and the writer. Field conditions alone do not consistently permit such effective separation of easy-bolting from nonbolting types.

The facts that thermal induction is greatly retarded at temperatures near 0° C., that the initial rate is more rapid at 6° to 9°, and that the rate of reversal of the process is greatly accelerated at about 25° suggest an explanation for the influence that certain cultural practices, designed to modify the thermal environment, also have on bolting (*9*, *16*) and afford a better basis for devising methods to influence bolting. In beets grown for seed, complete bolting is desirable and practices favorable to it should be used; but in beets grown for sugar, bolting is objectionable and practices unfavorable to its occurrence should be followed.

SUMMARY

A measurement of the relative influence of different storage temperatures in inducing reproductive development in sugar beets was made by conducting bolting tests in a warm environment under long photoperiods. In general, beets bolted more rapidly and in larger numbers after storage at 6° to 9° C. than after storage at cooler temperatures for similar periods. Storage of beets at temperatures near 0° induced little change in the rate and percentage of plants bolting, indicating that the processes involved in thermal induction are nearly arrested

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during such storage. Reversal of thermal induction occurred in beets that had been thermally induced previously, when those beets were stored at relatively warm temperatures (11° to 26°). The rate of both thermal induction and reversal of the process increased as the temperature was raised, the temperature coefficient of reversal being about 3.3 between 13° and 19°. The maximum amounts or degrees of thermal induction possible at the various temperatures within the favorable temperature range appear to form a gradient. Apparently there is continuity in the rate of any thermally induced change.

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ENVIRONAL FACTORS AFFECTING DOWNY MILDEW OF CABBAGE [1]

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INTRODUCTION

Downy mildew (*Peronospora parasitica* (Fr.) Tul.) has been reported as a serious disease of the kohl group of crucifers (*Brassica oleracea* L.) in Europe (*2*, *12*, *15*),[2] India (*3*, pp. 297–300), South Africa (*6*), Australia (*17*), and the United States (*5*, *8*, *11*, *13*, *18*). The disease on cabbage (*B. oleracea* var. *capitata* L.) has several phases, the severity of each depending upon current weather conditions. It commonly causes severe damage to seedlings, particularly during winter months, in the southeastern part of the United States (*4*, *7*, *13*, *21*).[3] Ordinarily the fungus continues to develop after transplanting but it is usually of minor importance at that time. As the crop approaches maturity under environmental conditions favorable to the mildew fungus, damage to the head may result. Bacterial soft-rot organisms may enter mildew lesions and cause damage in transit, while systemic invasion of heads in storage may lead to a dry-rot decay (*16*, *19*).[4]

The investigation reported herein is concerned with the host range of the physiologic race of the causal organism occurring on *Brassica oleracea* in the United States and with the effect of environmental conditions, particularly temperature and host nutrition, upon development of the disease in cabbage seedlings. The studies were carried on in the greenhouse at Madison, Wis.

HOST RANGE OF THE PATHOGEN

The problem of distinguishing between the various forms of *Peronospora* which occur on members of the Cruciferae has concerned mycologists since the first description was made in 1796 by Persoon (*9*) as *Botrytis parasitica* Pers. on *Capsella bursa-pastoris* (L.) Medic. Fries (*9*) in 1849 transferred the fungus to the genus *Peronospora*, which had been established in 1837 by Corda in his description of *P. ramicis* Corda. Wilson (*22*) suggested a division of *P. parasitica* into three species on the basis of morphological differences in the conidia. Gäumann (*9*, 10) demonstrated that spore size alone was

[1] Received for publication March 15, 1944.

[2] Italic numbers in parentheses refer to Literature Cited, p. 80.

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not an adequate means of separation of species in this group, but, as a result of further morphological studies and cross-inoculation experiments on over 100 species of the Cruciferae, he published an extensive report on the physiologic races of *P. parasitica* and proposed 52 new species. In view of the uncertainty with which races of this fungus can be distinguished on any other basis than their host relations, the writers have retained the binomial *Peronospora parasitica* (Pers.) Fr.

Collections of the fungus on cabbage were secured from Florida, Louisiana, Texas, and Wisconsin. They were first inoculated to members of *Brassica oleracea*, including red and green varieties of cabbage, Brussels sprouts (*B. oleracea* var. *gemmifera* DC.), cauliflower (*B. oleracea* var. *botrytis* L.), broccoli (*B. oleracea* var. *botrytis* L.), kohlrabi (*B. oleracea* var. *gongylodes* L.), and collard (*B. oleracea* var. *viridis* L.). All of these were very susceptible to each collection of the fungus. When each collection was inoculated to radish (*Raphanus sativus* L.), turnip (*Brassica rapa* L.), rutabaga (*B. campestris* var. *napobrassica* (L.) DC.), rape (*B. napus* L.), charlock (*B. arvense* (L.) Ktze.), and black mustard (*B. nigra* (L.) Koch) lesions were produced in the form of necrotic flecks, but no spores were produced. In the case of garden cress (*Lepidium sativum* L.), stock (*Matthiola incana* var. *annua* (L.) Voss), wallflower (*Cheiranthus cheiri* L.), arabis (*Arabis alpina* L.), shepherds-purse (*Capsella bursa-pastoris* (L.) Medic., and white mustard (*B. hirta* Moench (*B. alba* (L.) Rabenh.)), no visible signs of infection whatsoever were observed. It appeared, therefore, that the four collections belonged to a single race and that this race was confined in its successful pathogenicity to *B. oleracea* in the list of possible hosts tested. While other possible host species should be tested, it is evident that there is as yet no basis for considering wild hosts as a source of inoculum of the cabbage downy mildew organism in America. Elimination of cruciferous weeds is, therefore, not a well-founded control measure. The collection from Wisconsin was used for further experiments described in this paper.

TEMPERATURE RELATIONS OF THE FUNGUS

SPORULATION

Leaves selected late in the afternoon from inoculated plants, 2 days after symptoms had become visible but before sporulation had occurred, were chilled by placing them at 4° C. for 1 hour. They were then sprayed with distilled water from an atomizer, and six leaves, selected at random, were placed in each of seven moist chambers at 4°, 8°, 12°, 16°, 20°, 24°, and 28°, respectively. After approximately 12 hours, sporulation had occurred at temperatures of from 4° to 24°, the most abundant development of spores occurring at 12° and 16°. At 36 hours, sporulation was still greatest at 12° and 16° and nearly as profuse at 8°, but it was sparse at 4°, 20°, and 24°, and at 28° there was none. A repetition of this series with leaves on which the lesions were somewhat older gave similar results. Other observations in the greenhouse confirmed the evidence that best sporulation occurs at 8° to 16°, and it was noted further that from the lesions at these temperatures the fungus continued to sporulate longer than at other temperatures.

GERMINATION OF CONIDIA

Conidia were collected in the morning from leaves which had not previously sporulated and which were from plants that had been placed in a moist chamber the previous afternoon. This method was most satisfactory since it provided a crop of newly formed conidia. Fresh spores were desirable because it had been observed that they were extremely sensitive to aging and to desiccation. Suspensions in redistilled water were made by removing the conidia from the sporulating lesions with a camel's-hair brush. Glass slides were treated for 24 hours in a solution of potassium dichromate in concentrated sulfuric acid, washed in distilled water, boiled for a few minutes in a solution of sodium bicarbonate, washed in several changes of distilled water, and stored in distilled water until needed. Small, moist chambers lined with water-saturated absorbent cotton were maintained in incubators at the various temperatures to be studied. Slides to be used were placed across glass rods in the bottom of each chamber. After at least 12 hours had been allowed for the moist chamber and slides to adjust to the temperature of the incubator, drops of freshly made spore suspension were placed on each slide. With these precautions little or no condensation on the slides occurred, and the drops of spore suspension remained for a week or 10 days without an appreciable change in volume. The constant-temperature incubators used were adjusted to 4° to 6°, 8° to 10°, 12°, 16°, 20°, 24°, and 28° C. A slide was taken from each chamber at various intervals, and a drop of osmic acid was added to the drops of spore suspension to kill and to fix spores and germ tubes immediately. At least 500 spores were included in each determination. The temperatures inside and immediately outside of each chamber were recorded with each determination.

Several experiments were run with similar results. The data from a typical one are presented in figure 1. A high percentage of germination occurred promptly at all temperatures between 6° and 16° C. Germination was very much reduced at 20°, very meager at 24°, and none occurred at 28°. It may be seen, however, that the initiation of germination was decidedly the most rapid between 8° and 12°, where nearly 60 percent had germinated within 3 hours, while less than 20 percent germination was recorded at all other temperatures at this interval. This is, of course, a very important factor when the period of high humidity conducive to germination is short. At intervals above 8 hours there was little difference in the percentage of total germination at temperatures of 16° or below. In fact, there was a tendency for the highest percentage of germination to occur at the lowest temperature, which in this case was about 6°.

PENETRATION

Leaves of comparable ages were picked from young cabbage plants, and six, selected at random, were placed in each of six moist chambers, which were placed in incubators adjusted to 4°, 8°, 12°, 16°, 20°, and 24° C., respectively. After 24 hours several drops of spore suspension were placed on the lower side of each leaf. Leaves were removed frequently from each chamber and examined for the percentage of germination, formation of appressoria, penetration, col-

lapse of conidia, and formation of haustoria. The results are given in figure 2. Germ tubes appeared first at 12° and 16° and next at 8°. Appressoria formed first at 16° and next at 12°, 20,° and 24°. Penetration occurred first at 16°, next at 20° and 24°, and spores collapsed first at these three temperatures. Haustoria formed first 5 hours

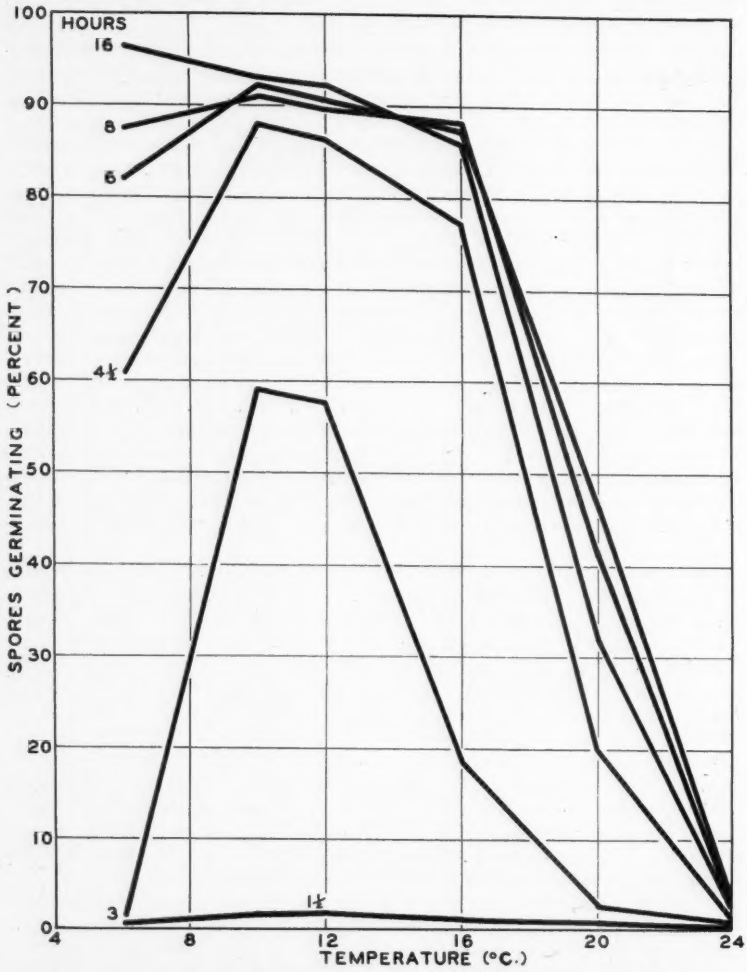

FIGURE 1.—Effect of time and temperature on germination of conidia.

after inoculation at 16° and 20°, and an hour later at 24°. Thus, while temperatures between 8° and 12° showed the most rapid germination as measured by percentage of spores germinating, the process of appressorial formation and penetration was optimum at

16°. Moreover, penetration and development of haustoria proceeded more promptly at 20° and 24° than at 12°, and the progress was decidedly delayed at 8° and 4°. After 22 hours the haustoria were

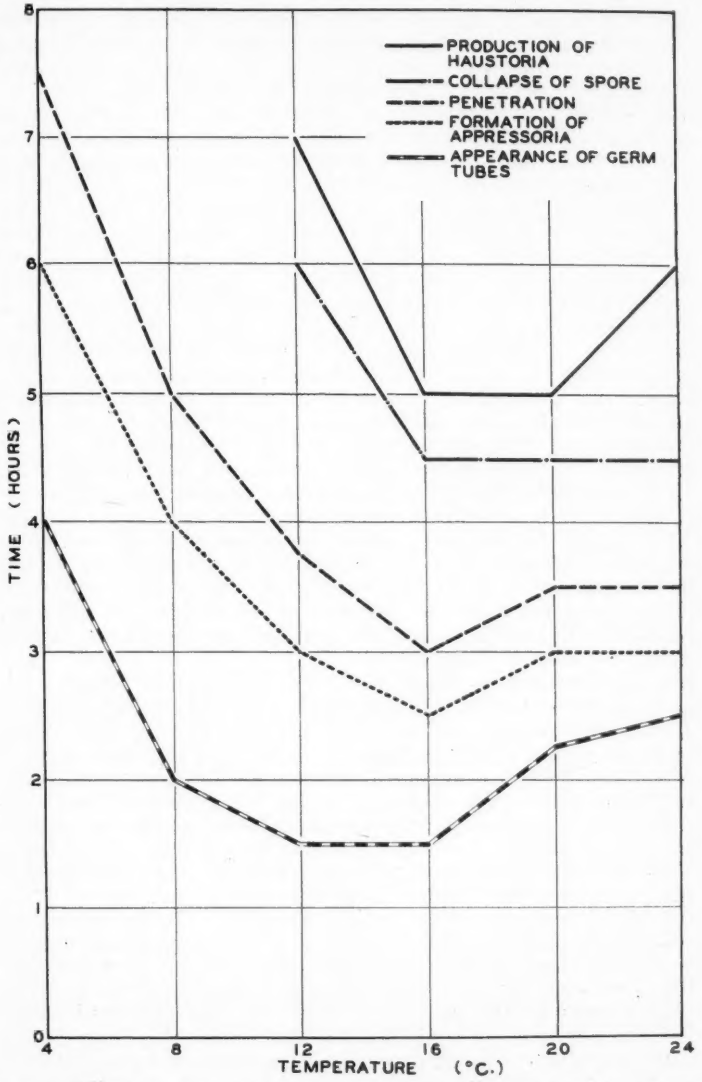

FIGURE 2.—Effect of temperature upon penetration of fungus and development of haustoria.

most abundant, and the diameter of the mycelium was greatest at 16°. The development of the fungus was next most vigorous at 20°.

RELATION OF TEMPERATURE AND HUMIDITY TO DISEASE DEVELOPMENT

CONSTANT TEMPERATURES AND HUMIDITIES

The development of mildew was studied first at constant temperatures and high humidity on young seedlings. Four flats of plants just showing a third leaf were inoculated with a suspension of spores. After 20 hours at 15° C. in a moist chamber, chlorotic spots with faintly flecked centers marked the points of infection on the first and second leaves. At this stage one flat each was placed in greenhouses at 16°, 20°, 24°, and 28°, respectively. Three days after inoculation cotyledons at 24° had begun to wilt, and leaf lesions were distinctly more advanced than at 20° or 28°; little progress was evident at 16°. At 4 days the cotyledons at 24° had become desiccated, and lesions were more pronounced on the hypocotyl and leaves. The disease continued to progress most rapidly at this temperature, followed in descending order of severity by temperatures of 28°, 20°, and 16°.

The experiment was repeated with potted plants having six to eight leaves. After inoculation and a 24-hour incubation in a moist chamber at 15° C., some plants were placed on an open bench in relatively dry atmosphere, and others were kept under a cloth moist chamber in which the air was kept saturated by means of a fine spray for an atomizer. The temperatures at high humidity were 12°, 16°, 20°, and 24° C., respectively, while those at low humidity were 16°, 20°, 24°, and 28°. Symptoms were evident at low humidity at 20°, 24°, and 28° on the fourth day after inoculation and on the fifth day at 16°. Again the disease progressed most rapidly at 24° and nearly as rapidly at 28° while the affected leaves dropped promptly at these high temperatures. At high humidity the disease developed most rapidly, and sporulation was abundant at 24°, while at this temperature and at 20° the tissue on which sporulation occurred was invaded promptly by secondary organisms.

In a third experiment plants at the fifth-leaf stage were used. The same temperatures at low humidity were provided; at high humidity the temperatures were 12°, 16°, 19°, 23°, and 28° C. The results are presented graphically in figure 3. Since the presence of spores actually marks the completion of the cycle in the host, their appearance is the most suitable character for use in determining the incubation period. Furthermore, the development of symptoms may be influenced by the effect of relative humidity, sunlight, soil moisture, and other factors on the host, while sporulation is a direct indication of the progress of the fungus. The disease appeared first at 24° at low humidity and next at 23° at high humidity. Initial sporulation appeared first at 23° at high humidity. The lesions at 20° and 24° were darkest in color; those at 28° showed a pronounced yellow halo.

The three experiments demonstrated that progress of the fungus in the host tissue was most rapid at about 23° C. At high humidity the cycle from conidium to conidium was shortest at this temperature, although, as shown earlier, sporulation was most abundant at from 8° to 16°. The appearance of symptoms was also most prompt at 23° or 24°. The fact that the first signs appeared some 12 hours earlier at low humidity is due to the fact that invaded tissues collapse more rapidly at low than at high humidity and the lesions thus become visible more promptly.

ALTERNATE LOW AND HIGH HUMIDITY

Natural climate often consists in alternating periods of high and low humidity. To approximate such a condition with controlled temperature, cabbage plants selected for uniformity of size and vigor were inoculated in the moist chamber at 16° C., and 5 days later when

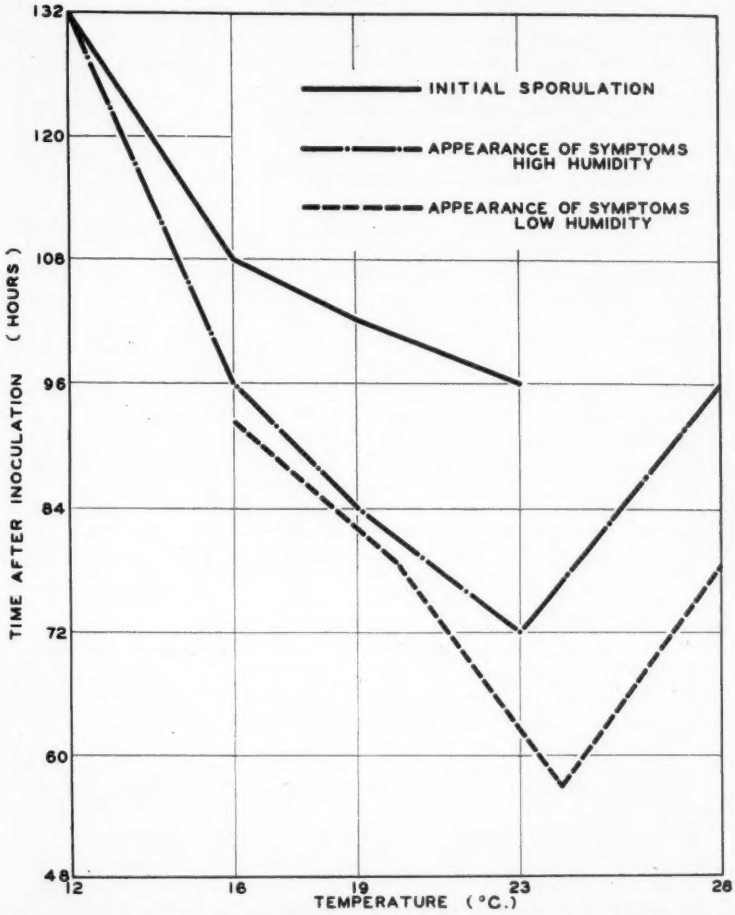

FIGURE 3.—Effect of five different temperatures on the initial sporulation of fungus at high humidity and upon initial appearance of symptoms at low and at high humidity.

heavy and uniform infection became evident, they were transferred to greenhouses at 16°, 20°, 24°, and 28°. Twelve plants were placed in a moist chamber and six on an open bench in each house. The plants in the moist chambers were subjected to alternate wet and dry periods of 24 hours each. During the periods when the chambers

were sprayed with water, their temperatures ran 4 to 5 degrees lower than that of the respective houses in which they were kept, while during the dry periods the temperatures in the open moist chamber and in the remainder of the house were approximately equal. This treatment continued over a period of 4 weeks. To simulate natural conditions and bring about spore dispersal, the atomizer spray in each moist chamber was directed at the plants.

At 28° C., sporulation was abundant in the moist chamber during the first two treatments but rapidly decreased in subsequent moist periods as the infected leaves dried and fell. After 4 weeks a few small static lesions from the original inoculation remained, but no new lesions were evident. The plants on the open bench had lost all infected leaves. At 24° sporulation also decreased rapidly as early defoliation took place. A small amount of secondary infection was evident after 10 days, but at the end of 4 weeks there were no additional lesions and the spots resulting from secondary infection did not produce spores. Some remaining lower leaves showing original infection produced a few conidiophores at the margins of old lesions. The plants on the bench also retained leaves showing static lesions. At 20°, sporulation rapidly declined after the first week. There was, however, a considerable amount of secondary infection producing lesions with normal sporulation. All plants retained leaves infected by the original inoculation, and in some of these lesions the fungus sporulated to a slight degree along their margins. The plants on the bench retained fewer of the originally infected leaves. At 16°, sporulation was abundant up to the time the plants were discarded, spores being produced on new as well as old lesions. Thus, the disease was most active by far at 16° because of the presence of new lesions and the continued activity of old lesions. The prolonged sporulation which followed the alternate dry and moist periods might have been due in part to the low temperatures during the moist treatments.

Upon the repetition of this experiment, but with a moist period of 48 hours only once a week, the results were essentially the same. Secondary infection was again sparse at the three highest temperatures although sporulation was uniformly heavy at first. The inoculum was soon depleted by the rapid drying of the infected leaves before additional leaves had reached stages suitable for infection. Lesions which did occur at 24° and 28° C. on young leaves were small and inactive. At 16° new infection had taken place, and old lesions were still active.

The effect of temperature at alternate periods of low and high humidity was similar to that at constant humidities. As the temperature was raised, the rate of mycelial growth and development increased up to 24° C. but was retarded somewhat at 28°. However, temperatures of 20° and above were less favorable to disease development since the active sporulating period of leaf lesions was greatly shortened and germination of spores was reduced. It was apparent that at 20°, 24°, and 28° the host rapidly outgrew the effects of infection, for as the infected leaves quickly desiccated they became ineffective as sources of inoculum. While the fungus developed more slowly at lower temperatures (12° and 16°), the period of effective sporulation was greatly lengthened, and the reduced growth rate of the host permitted heavier and more effective infection.

The alternation of periods of high and low humidity had little effect on the rate of desiccation of lesions at 24° and 28° C. However, at 16° the treatment successfully maintained the disease. It seems probable that the early recovery of plants from the effects of the disease when temperatures were above 20° was in part the result of the greatly shortened period of activity of the fungus in the lesions and the increased growth rate of the host.

RELATION OF HOST NUTRITION TO DISEASE DEVELOPMENT

The reports of increased or reduced susceptibility of cabbage and other crucifers to downy mildew resulting from variation in host nutrition are all based on field observation. Quanjer (*15*) stated that cauliflower plants showing marked potash deficiency symptoms were severely attacked by *Peronospora parasitica*, while those fertilized with potash were but slightly attacked. Chupp (*4*) found considerable reduction of tipburn but a marked increase in mildew where potassium had been added. Fertilization with nitrates also increased the severity of mildew, but phosphates seemed to decrease the amount of disease. Chupp pointed out that the experimental data were too limited to warrant definite conclusions but that the differences in disease development were striking. Townsend (*20*) obtained results which indicated that cabbage plants grown in soil treated with phosphorous were more subject while those in soil treated with potash were less subject to the disease than plants grown on unfertilized soil. Brejneff (*1*) reported very little difference in downy mildew incidence on crucifers grown with various applications of fertilizers. De Bruyn (*2*) found that nutrition had little effect on susceptibility except where it influenced the vigor and condition of the leaves. Chlorotic leaves approaching senescence were much more susceptible than vigorous ones, although plants were more susceptible during the summer when light was stronger and of longer duration. She concluded that the influence of soil fertility was secondary to that of the vigor of the foliage.

The conflicting evidence just cited indicated that a profitable study might be made of the effect of nutrition on downy mildew. A constant drip method, already described by Pryor (*14*), for applying nutrient solutions to pure quartz sand was adopted for the present investigations. Each series of plants was given 8 different nutrient treatments: Excesses and deficiencies of nitrogen, potash, and phosphorous; deficiency of sulfur; and a balanced nutrient. Cabbage seedlings were grown to the first-leaf stage in sterile quartz sand and 20 were transplanted to each pot. Three or 4 pots were used in each treatment. When the plants were 7 to 9 inches tall and showed from 8 to 10 leaves, they were inoculated simultaneously in a large chamber and returned after 24 hours to the drip system. Disease readings were taken after symptoms had fully developed. The number of lesions on mature leaves and their relative size were estimated and rated according to severity in classes ranging from 1 to 10, and a disease index was obtained by multiplying lesion-spread by lesion-number ratings. A sporulation index was used to indicate the proportion of the lesions which sporulated after the period allowed for development. A plant-size index was also employed with 1 representing the smallest size of plant in any given series and 10 the largest.

The plants in the first experiment were placed in the moist chamber on the morning of March 2 and were inoculated at 12° to 15° C. with a uniform suspension of freshly produced spores late the same evening. The moist treatment was continued until the following afternoon, and additional 24-hour moist treatments were provided on the third, fifth, and ninth days. The readings were made on the tenth day following inoculation when the more severely diseased leaves were almost entirely permeated by the fungus. The second experiment, started on May 15, was conducted similarly except that the inoculation temperatures were between 15° and 17°. This series was reinocu-

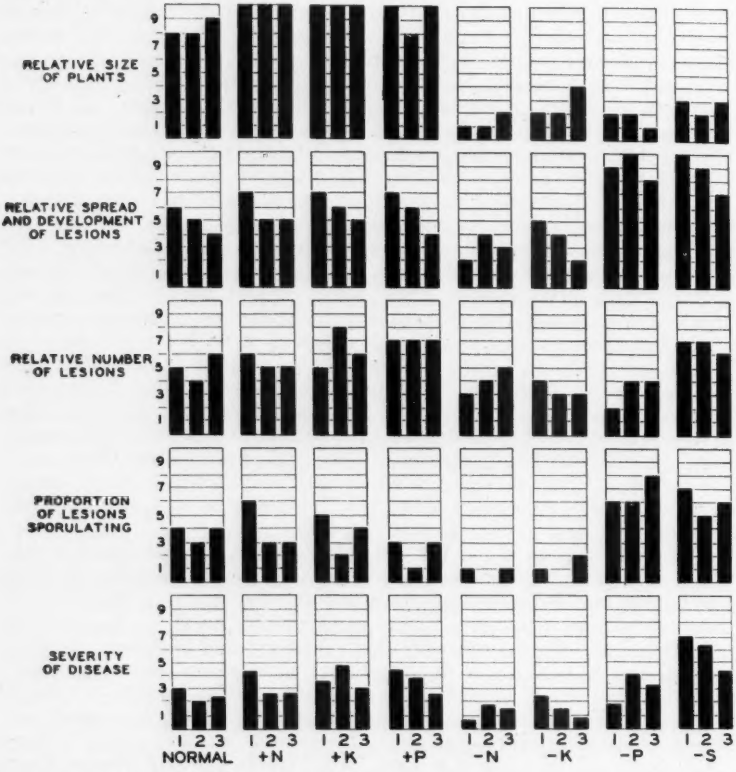

FIGURE 4.—Graphic summary of infection by and development of *Peronospora parasitica* on cabbage plants grown in sand culture supplied with various nutrient solutions.

lated on the second day, and a moist treatment was provided on the ninth day. Results were recorded 10 days after inoculation. In general, the number of lesions was slightly lower in this experiment than in others, probably because of the low vitality of the spores in the suspension used for inoculation. The third experiment was run during July when temperature was difficult to control. The moist

chamber varied between 15° and 18° during the 20-hour inoculation period. The day temperature of the greenhouse was about 32° and early drying of the diseased leaves occurred. Infection was heavy and records were taken 8 days after inoculation.

Examination of the data from these three experiments reveals differences between the various nutrient treatments both in number and type of lesions. The results are presented graphically in figure 4. While the plants in the deficiency solutions were very stunted, certain differences appeared consistently. The relative number of lesions developing on the plants deficient in N, K, and P was considerably less than on plants deficient in S and on those having an excess of N, K, and P. A difference in amount of bloom on the leaves might explain part of the variation. In the −P and −S series the lateral spread of the fungus and the amount of sporulation were markedly greater than in the plants of comparable size of the −K and −N series, and noticeably more than in the larger plants of the series with normal nutrition or with excessive amounts of N, P, or K. In the −P series the greater development of the fungus as compared with that in other series was associated with greater thickness of the crinkled, stunted leaves.

Since there was reduction in size of plants with all deficient solutions there was no correlation between susceptibility and vigor as indicated by size or rate of growth. In general, lesions developed slowly on immature leaves and very rapidly on senescent leaves. Physiologically old leaves tended to develop more rapidly in −P and −S solutions than in −N and −K solutions and this seemed to account for the greater spread of lesions and more abundant sporulation on plants in the first two solutions.

The previously noted relation between deficient chlorophyll and susceptibility was not apparent in these experiments. Leaves in the −S and −N series were quite yellow, those in the −K series were bluish green, and those in the −P series were light green. The plants grown with excessive concentrations of N, P, or K showed little or no difference in either number or spread of lesions.

A fourth experiment was conducted in which the concentration of the basal solution was increased 10 times and 30 times. On June 1, when the plants had from six to eight leaves, they were inoculated. Little difference in vigor of plants at the three concentrations was evident until the time of inoculation, when plants in the highest concentration had attained growth beyond the optimum. Infection was uniformly good, but the disease developed most severely on the plants at the highest salt concentration. There was little difference in disease development between the lowest and the intermediate concentrations.

DISCUSSION AND SUMMARY

The severity of downy mildew on young cabbage seedlings is greatest in areas in the United States where the plants grow in seedbeds throughout protracted cool periods during which relative humidity is high. According to Eddins (7), the disease is most destructive in Florida when the temperature ranges between 10° and 15° C., and when the plants remain wet until midmorning for 4 days. The studies reported herein show that the fungus sporulates most readily at a

range of 8° to 16°, the conidia germinate most rapidly at 8° to 12°, and penetration of the host occurs most rapidly at 16°. Thus, these stages of the life cycle of the fungus which are essential to the development of inoculum and to infection are favored by relatively low temperatures.

After invasion of the host, haustoria grow most rapidly at 20° and 24° C. When disease development was studied at a range of temperatures, it was found that the symptoms developed most rapidly at 24°. When humidity was high, the lesions which developed first at this temperature also sporulated first. However, the disease ran its course most rapidly at the temperature most favorable for mycelial spread within the host. Sporulation and reinfection were limited at 24° and at 28°, and an increased growth rate of the host resulted in more rapid maturation and dropping of the lower leaves. At the low temperature (16°) the growth of host and fungus was slower, but sporulation and reinfection were much more pronounced. Here the fungus was most prolific, and the disease developed most profusely.

It is thus apparent that the low optima for sporulation, germination, and penetration are more important to disease development than the higher optimum for growth of the fungus. The severity of the disease at about 10° to 15° seems to be best explained by the effect of temperature upon production of inoculum, spore germination, and infection.

The confusing observations recorded in the literature on the effect of fertilizers on the development of mildew were not cleared up by the study of disease development on plants grown in a range of controlled nutrients. In fact, the results would indicate that little benefit could be expected in the control of mildew through adjustment of fertilization of seedbeds.

The commonly cited recommendation of eradication of cruciferous weeds to control this disease is of no value in view of the fact that the collections made of the fungus from widely separated localities in the United States were apparently all of one physiologic race. This race was confined in its pathogenicity to members of *Brassica oleracea*, of which none grow in the wild state in this country.

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DIFFERENTIAL BACTERICIDAL ACTIVITY OF BOVINE SERUM TOWARD STRAINS OF BRUCELLA ABORTUS OF HIGH AND LOW VIRULENCE [1]

By M. R. IRWIN, *Professor of genetics, Wisconsin Agricultural Experiment Station, and cooperative agent, Bureau of Animal Industry, Agricultural Research Administration, United States Department of Agriculture*, and B. A. BEACH, *Professor of veterinary science, Wisconsin Agricultural Experiment Station* [2]

INTRODUCTION

The discovery by Bordet (*3*) [3] that the bactericidal action of serum depends upon two elements, now known as immune body and complement, was followed by intensive study of these phenomena. Much of the earlier literature has been reviewed by Muir and Browning (*14*), and an extensive reference to later work has been given by Dingle, Fothergill, and Chandler (*5*). The role of complement in immunological phenomena has been reviewed in the excellent treatise by Osborn (*15*).

Perhaps the majority of the studies have been made with the primary purpose of explaining the mechanism of immune processes. There have been some, however, which attempted to correlate the bactericidal or virucidal activity of whole blood or serum with variation in the virulence of a pathogen, or to determine whether such activity was associated with resistance or susceptibility of a host to a particular infectious agent. For example, a report by Malone, Avari, and Naidu (*13*) has shown that in the black rat (*Rattus rattus*) there was a significant and positive correlation between the bactericidal power of whole blood and resistance to *Bacillus pestis*. In an attempt to determine the nature of inherited resistance to *Salmonella enteritidis* in the laboratory rat (*R. norvegicus*), Irwin and Hughes (*11*) obtained a similar positive correlation between antibacterial action and resistance. Furthermore, preliminary results by these authors (*10*) suggested that the difference between individuals (in rats) in antibody content was determined in part by heritable factors. Bull and Tao (*4*) report their own and cite other studies as indicating an association of bactericidal activity of whole blood and resistance in rabbits, and in chickens as compared with rabbits, to pneumococcal infections. The experimental results of Silverthorne (*17*) and Silverthorne and Fraser (*18*) indicate that the bactericidal power of whole blood of human beings and guinea pigs against various strains of meningococci is correlated with the virulence of these organisms and with protection after immunization.

Studies on the relation of the age incidence in human beings to the antibacterial activity of whole blood against pneumococci were reported by Sutliff and Finland (*19*) and against *Haemophilus in-*

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[2] The writers gratefully acknowledge their indebtedness to Warren G. Black, formerly a research assistant, who aided greatly in the technical aspects of this investigation.

[3] Italic numbers in parentheses refer to Literature Cited, p. 90.

fluenzae by Fothergill and Wright (*7*). The findings of these authors were in agreement in showing that the bactericidal power of the blood of children at an early age was relatively low or absent, and that there was an increase of this activity with advance in age. The technique has been widely employed in studies of pneumococcal infections. Ward (*20*) found that the whole blood of individuals (human) varied considerably in activity toward different types of pneumococci. Dozois, Seifter, and Ecker (*6*) have stated:

> Although much of the work on bactericidal and hemolytic activities is contradictory, it has been firmly established that these activities depend on the interaction of a thermostable antibody and a thermolabile complement. Only the thermostable antibody is increased by immunization. Neither substance by itself has bactericidal or lytic effects.

It has previously been shown by Irwin and Ferguson (*9*) that bactericidal antibodies ("bactericidins") of high titer were present in the serums of cattle which had recovered from an induced infection of *Brucella abortus*, and whose serums had ceased to react in agglutination with the organism beyond the range of serums from normal cows. The studies reported in this paper were made to test whether (1) the serums of cows vaccinated with strain 19 of *Br. abortus* would exhibit parallel phenomena, and (2) whether there would be a differential activity of such serums toward strain 19 and one of greater virulence.

MATERIALS AND METHODS

On September 23, 1940, 15 cows varying in age from a minimum of 18 months were vaccinated subcutaneously with 5 cc. of *Brucella abortus*, strain 19. These animals were offspring of the cows that had made up herd 3, which has been previously described (*1*, *2*). They had been bred 1 to 3 months prior to vaccination.

Previous experience [4] in this laboratory in measuring the bactericidal effect of serums which had a titer of agglutinating antibodies beyond 1:100 for *Br. abortus* had shown that a definitely higher titer of antibodies with antibacterial action ("bactericidins") than of agglutinating antibodies could usually be demonstrated in such serums. However, since the serum of infected animals from whose blood the agglutinins had practically or entirely disappeared still contained demonstrable bactericidins for *Br. abortus*, as previously reported (*9*), comprehensive tests on the bactericidal activity of the serums of these vaccinated animals were delayed until the agglutinating titer of their respective serums was usually 1:100 or less. The tests to be reported in this paper were made on the serums of the animals between 6 and 18 months after the vaccination.

The organisms used in these tests were cultures of *Br. abortus* of strain 19 and a virulent strain, No. 1242[5]. Shortly after the experiments were started, tests [6] for the virulence of strain No. 1242 were made on two isolates from single colonies, by the usual method of injection into guinea pigs. Each of six animals in the group injected with organisms of one or the other of the isolates showed a high titer of agglutination and definite lesions, but of varying degrees, in the

[4] By the authors and Dr. L. C. Ferguson.

[5] Both strains were obtained through the courtesy of Drs. A. B. Crawford and Adolph Eichhorn of the Animal Disease Station, Bureau of Animal Industry, U. S. Department of Agriculture.

[6] The writers are indebted to Mrs. Mildred M. Johnson for making the tests of the virulence of these isolates.

spleen. These results were taken as a reasonable index of the virulence of the isolates. Care was exercised in culturing both strains to avoid the propagation of variants.

Previous trials of the bactericidal activity of whole blood, plasma, serum, and white blood cells alone have shown that the primary, if not the entire, bactericidal properties of cattle blood toward *Br. abortus* are found in the serum (*8*). (White blood cells alone or in combination with the red cells had no bactericidal effect whatever on *Br. abortus*. As previously stated (*8*), serum alone, even from cows whose whole blood had very little antibacterial activity, showed more bactericidal action toward *Br. abortus* than did whole blood.)

Essentially the same technique was employed in making the tests of the bactericidal activity of serum from the various animals as has been previously described (*8*, *9*, *16*). Blood was taken under aseptic conditions from the jugular vein of the animals into tubes, and allowed to clot. The tubes usually stood at room temperature for about an hour, and were then stored in a refrigerator until the following morning. Beef serum diluted more than 1:5 usually shows very little if any antibacterial action for *Br. abortus*, unless complement from beef serum is added. As has been reported by Shrigley and Irwin (*16*), complement from guinea pigs or rabbits is incapable of activating beef serum against *Br. abortus*, so the complement in these tests was obtained from cattle not infected with *Br. abortus* (i. e., "normal" animals). Considerable differences in activity of the complement from different individuals have been encountered, and, insofar as was possible, serum from one or the other of two individuals was used as the source of complement in the tests. The complement was prepared by absorbing serum from defibrinated blood with heat-killed cells of *Br. abortus* (0.3 cc. of bacteria per 10 cc. of serum), at 0° C. for 20 to 30 minutes. The antibodies, but not the complement, of the serum are absorbed at this temperature. The absorption was usually done in late afternoon, and the complement was stored overnight in a refrigerator after being removed from the bacterial serum mixture. Centrifugation of the mixture was more quickly done with an "angle centrifuge" than with the usual type.

In the bactericidal set-up there was a series of tubes (pyrex) for each serum and the different dilutions of each serum to be tested. These tubes contained, individually, 0.05 cc. of a decimal dilution of a standardized suspension of a 36- or 48-hour culture of *Br. abortus*, either strain 19 or 1242. The organisms were washed off the slant in saline, and the volume was adjusted to correspond approximately in number with a standard suspension. From this, the first dilution was made, 10^{-1}. The successive dilutions are termed 10^{-1}, 10^{-2}, . . . 10^{-7}, respectively, according to general usage. The dilutions were made in broth so as to avoid any antagonistic action of saline on the organisms. To each of the tubes in the series was then added 0.3 cc. of serum, or of the dilutions of serum; to the tubes containing diluted serum was also added 0.2 cc. of the complement. Following incubation at 37° C. for 22 to 24 hours, the contents of each tube were poured into a pyrex Petri dish with fluid tryptose agar and incubated 5 or 6 days. The number of colonies in each plate was then compared with that in the bacterial controls. As a control in each set-up on the activity of the complement, 0.3 cc. of serum which had been heated at 56° C. for 30

minutes, complement (0.3 cc.) alone, and the combination of heated serum (0.3 cc.) and complement (0.2 cc.) were tested in the same manner as outlined above for the serum. Only those tests in which the complement restored bactericidal activity to heated serum approximately equivalent to that of the undiluted serum were considered to be satisfactory and have been included in the data to be presented.

For the bacterial control, 0.05 cc. of each of the bacterial dilutions was plated out directly in fluid tryptose agar, and the number of colonies in the two highest dilutions was counted after incubation. Usually duplicate samples were made of the two highest dilutions. Also, 0.05 cc. of each of the three or four highest dilutions of bacteria was placed in broth, incubated, and then plated with the others. The colonies from the highest dilution of bacteria (10^{-7}), when grown in broth, were always too numerous to be counted, as compared with an average number of 10 in the control.

Thus in the test as a whole, the ability of the organisms to grow was determined by seeding from each of the higher dilutions into broth, and the approximate number from each dilution placed in the respective tubes to be tested with the serum and its dilutions was measured by the bacterial control. The number of organisms in the series of plates testing the bactericidal activity of heated serum was usually slightly less than that of the bacterial control, indicating probably a minimum inhibition of growth by the heated serum. If there was little or no bactericidal activity in the tests on the complement alone, it was concluded that the absorption of antibodies was adequate and the addition of complement to the various dilutions of serum did not by itself produce any antibacterial activity. All these factors were satisfactorily controlled before the test at any time on any animal was considered to be adequate.

EXPERIMENTAL RESULTS

The antibacterial action of the serum and dilutions of serum was estimated by contrasting the number of colonies in the tryptose agar controls with those in the organism-serum mixtures. An index of the bactericidal activity of the serum, or dilution of serum, of any individual was calculated in the manner suggested by Mackie and Finkelstein (*12*), by subtracting the last (bacterial) dilution in which growth occurred in the serum mixtures from the last dilution in which growth occurred in the control. Thus, as is shown in table 1, the undiluted serum of cow No. 68 as a rule allowed slight growth of strain 19 in the –2 dilution, none in the –3. However, the bacterial control showed a mean number of colonies of between 10 and 20 in the –7 dilution, and the bactericidal index of these tests is then 5. The average number of colonies, in all the tests made, in the highest dilution (–7) of bacteria was between 10 and 20. That in the next lower dilution (–6) was between 100 and 200.

Thus, 0.3 cc. of serum from this normal animal was able to destroy somewhat more than 100,000 bacteria of strain 19, a bactericidal index of 5. As is shown in table 1, in the same number of tests, made at the same time, the bactericidal index toward the virulent strain (No. 1242) of *Br. abortus* was 4, or more than 10,000, but less than 100,000 organisms killed by the same amount of serum which destroyed more than 10^5 organisms of strain 19. The bactericidal

indices for the various dilutions of serum of cow No. 68 against strains 19 and 1242, respectively, as shown in the table, are as follows: For serum diluted 1:10, the index was 5 for strain 19, 2 for 1242; at 1:20, an index of 3 for each strain; at 1:40, 3 for 19 and 1 for 1242; at 1:80, 2 for strain 19 and 1 for 1242; at 1:160, 2 for strain 19 and none for 1242; and at 1:320, no definite antibacterial activity for either strain.

TABLE 1.—*Summary of several tests on the antibacterial action of the serum of a normal animal (cow No. 68) against 2 strains of Brucella abortus*

Serum	Strain of *Brucella* tested	Number of tests	Bacterial dilutions [1]						Bactericidal index
			10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	
Undiluted serum	19	9	+	0	0	0	0	0	5
	1242	9	++	++	0	0	0	0	4
Serum diluted:									
1:10	19	6	++	0	0	0	0	0	5
	1242	6	+++	+++	+++	++	0	0	2
1:20	19	3	+++	+++	++	0	0	0	3
	1242	3	+++	+++	++	0	0	0	3
1:40	19	6	+++	+++	+	0	0	0	3
	1242	6	+++	+++	+++	++	+	0	1
1:80	19	3	+++	+++	++	+	0	0	2
	1242	3	+++	+++	+++	+++	++	0	1
1:160	19	6	+++	+++	+++	+	0	0	2
	1242	6	+++	+++	+++	+++	+++	+	0
1:320	19	3	+++	+++	+++	+++	+++	+	0
	1242	3	+++	+++	+++	+++	+++	+++	0
Heated serum+complement	19	8	++	+	0	0	0	0	4
	1242	9	+++	+++	++	0	0	0	3
Average number of organisms in the dilutions of the bacterial controls			10^6	10^5	10^4	10^3	10^2	10	

[1] 0 indicates that there were no colonies in the combination of serum and bacterial dilution; + indicates the presence of a few colonies in some or all of the tests; ++, somewhat more colonies than indicated by +, but less than the bacterial control; +++, an appreciable number of colonies, but not necessarily as many as in the bacterial control.

Except for differences between individuals, the results observed with the serum of this animal are typical of those obtained with several other normal animals which have been tested in this manner. Usually little if any antibacterial activity toward either bacterial strain was shown by the serum of any normal animal at a dilution of 1:160. Occasionally an animal has been tested whose serum showed little or no bactericidal activity beyond a dilution of 1:10.

The reactivity of the serum of cow 68 was somewhat greater toward strain 19 than toward 1242 in the majority of these tests, as is indicated by the averages given in table 1. Only infrequently did the converse situation obtain. This differential action toward the two strains, themselves differing in virulence, was usually, but not always, encountered in repeated tests of a few other normal animals. In the majority of such tests, however, wherever there was a difference in reactivity of the serum toward the two strains of the organism, it was definitely in the direction of a greater number of organisms of strain 19 being acted upon than of the other.

According to these results, the serum of a normal animal rarely, if ever, exhibits bactericidal activity toward strain 19 of *Br. abortus*, or toward a virulent strain, beyond a dilution of 1:160. Repeated tests of such activity toward virulent strains were made at an earlier date on the serum of animals of herd 3, prior to their artificial exposure as previously described (*1*, *2*). The serums of only a few of the normal

animals of this group showed an antibacterial action at dilutions of 1:160, those of the majority had a maximum bactericidal titer of 1:40 or 1:80. Therefore, any increase in the titer of the bactericidins, following infection or vaccination, over the maximum observed for normal animals, may almost certainly be attributed to the stimulation induced by the infection or vaccination.

Data on tests performed with the serum of 12 vaccinated animals are presented in table 2. The digits given in the table represent the average bactericidal indices for the serum and various serum dilutions of the animals listed, toward each bacterial strain. The average indices are listed, rather than the results of single trials, as these should give for any individual a more accurate measurement of differential action toward the two strains of *Brucella* than any single trial. On the other hand, fluctuations in the titer of the bactericidins, particularly the anticipated decrease with time, would tend to be concealed in an average index, depending upon the time elapsing between tests on an individual.

TABLE 2.—*The average bactericidal indices, toward 2 strains of Brucella abortus, of the serums of 12 cows vaccinated at the age of 18 months or more with a culture of strain 19*

Cow No.	Strain of *Brucella* tested	Average indices [1] of bactericidal activity of—										Agglutination titer
		Undiluted serum	Serum dilutions at—								Heated serum + complement	
			1:10	1:40	1:160	1:640	1:1,280	1:2,560	1:5,120	1:10,240		
15E	19	4.2	4.7	3.3	2.7	3	3.5	4	2.5	.....	3.7	Partial at 1:100.
	1242	3	2.5	1.5	1.5	2.3	3	2.3	1.5	.....	2.5	
20E	19	6	5	4.3	4	4	4	2.7	1.7	.....	4.7	Partial at 1:100.
	1242	3.3	3	2.3	2.3	2.3	2.3	2	1	.....	2.3	
1F	19	5.3	4.7	3	5	3.7	4	3	3	.....	6	Partial at 1:50.
	1242	1.7	2	1.7	3	2.7	3	3	2	.....	4.3	
63F	19	6	5	5	4	4	.....	3	1	0	5	Partial at 1:100.
	1242	4	2	2	2	1	.....	1	0	0	3	
2g	19	6	4.5	4.5	4	3	.....	2	2	2	6	Partial at 1:50.
	1242	5	3	3	3.5	2.5	.....	0	0	0	4	
6g	19	4.7	3.3	2.7	3	2.7	.....	1.7	1	1	3.3	Slight at 1:200.
	1242	4	2.7	2.7	2.3	2	.....	1.7	1	0	2.7	
8g	19	6	5.3	4.7	4	2	2.5	1	.7	0	4.3	Slight at 1:100.
	1242	4	3	2.3	2.7	2.5	2	.5	.3	0	3	
11g	19	5	4.3	4.7	4	4.3	3.5	3.3	3	4	5	Slight at 1:25.
	1242	3	3.7	3.3	3	2.7	2	2.3	2	1	4	
25g	19	6	5.7	5	4	4	3	2	1	.....	3.3	Partial at 1:100.
	1242	3.7	2.7	1.7	2.3	2.7	2.7	1.7	.7	.....	3	
33g	19	6	5	4.5	4	3.5	3	2.5	2	.....	5.5	Slight at 1:50.
	1242	4.5	4	3	2.5	2	2	1	0	.....	3	
38g	19	5.7	5	4.2	3.5	3.5	4	3.5	1.7	.....	4	Slight at 1:100.
	1242	3.2	2	2	2	2	2	1.7	1	.....	2.5	
63g	19	5.7	5.3	4.3	4	3	2.3	.5	.3	.....	4.7	Slight at 1:50.
	1242	3.7	3.3	3	2.5	3	1.3	.5	0	.....	3.7	

[1] The digits represent the average index of bactericidal activity at the various dilutions of serum. (See text for explanation.) The average number of organisms of either strain at the highest dilution (10^{-7}) was between 10 and 20, and at the next highest dilution, between 100 and 200. Thus 0 indicates no organisms destroyed; 1, more than 10 but less than 100; 2, more than 100 but less than 1,000, etc., the indices corresponding approximately to the respective powers of 10 in number of organisms acted upon.

In these tests, the average number of organisms in the −7 dilution was 10 to 20, so an index of 1 for a particular serum dilution would represent no colonies present in that bacterial dilution. If colonies were present in this bacterial dilution of controls for heated serum and complement, the conclusion would be strengthened that the action of the serum dilutions and complement is definitely bactericidal. In the writers' opinion, however, an index of 1 is hardly large enough

to be considered as significant; an index of 2, indicating that 100 or more organisms were destroyed, may be taken as indicative of a slight, but appreciable, bactericidal action. The indices correspond to the respective powers of 10 in the minimum number of organisms killed. Thus, in addition to the number of organisms destroyed if the indices were 1 and 2, an index of 3 represents 10^3 or 1,000 as the minimum number killed, an index of $4=10^4$ or 10,000, etc.

In table 2, it may be seen that cow No. 15E showed a definite index of bactericidal activity in the undiluted serum and in the various dilutions through 1:5,120. These indices are averages of four tests, one made in June 1941, one in January and two in March 1942. Only in June 1941 and in January 1942 were the serum dilutions of 1:2,560 and 1:5,120 included in the tests on this individual. The bactericidal activity of this serum in the two later tests was markedly lower, more probably because of lessened activity of the complement than of decreased titer of antibodies, so that the average index of the dilutions up to and including 1:1,280 is low relative to that of the two higher dilutions. At these times of testing and at monthly intervals between, the serum of this animal regularly showed partial agglutination of *Br. abortus* in a dilution of 1:100.

Just how much difference in antibacterial action toward these two strains is required in order to be able to state definitely that there is significant differential action of the serum can hardly be answered. However, even small differences in differential activity, if repeatable over a series of tests, would certainly be considered significant. These tests should be considered on that basis.

The serum of this animal (15E) did not differ in the presence or absence of bactericidal activity toward the two strains, but rather in the number of bacteria of each strain acted upon at the respective dilutions. The general statement can be made in respect to the serum of this and all the vaccinated animals, as was done above for the serum of normal animals, that wherever there was a difference in antibacterial activity, it was practically always in the direction of greater activity toward strain 19.

The serums of the 11 other animals, listed in table 2, gave antibacterial activity comparable in general with the serum dilutions of 15E. Of those whose serums were tested at a dilution of 1:10,240, only two showed reactivity, viz, 2g and 11g. The serums of 20E, 1F, 63F, 6g, 25g, 33g, and 38g were reactive at dilutions of either 1:5,120 or 1:2,560, while the serums of 8g and 63g were reactive at a dilution of 1:1,280, and only slightly if at all, at 1:2,560. The highest agglutination titer at the time of these tests was a partial reaction at 1:200 for the serum of 6g. The lowest titers (partial reactions at 1:25 or 1:50) of agglutinins were displayed at the time of the tests by the serums of 1F, 2g, 11g, 33g, and 63g, while those of the others were partially reactive at dilutions of 1:100. There appears to be no significant correlation here between the titers of agglutinins and bactericidins.

From these results it may be stated that the serum of vaccinated animals with agglutinating titers at or below those defining "reactors," as shown by these data, nevertheless may have an appreciable titer of bactericidins for *Br. abortus* of either high or low virulence. These results substantiate those previously reported from this laboratory (*9*), which showed the presence of bactericidins in appreciable titer in

the serums of animals artificially exposed to a virulent strain of *Br. abortus* (*1*, *2*) and from whose serums agglutinating antibodies had disappeared. Although the average indices of bactericidal activity of the serum and serum dilutions of these animals did not *always* show a differential reactivity toward strain 19 and a virulent strain of *Br. abortus*, differential reactivity was shown in the *majority* of the dilutions of the serums of *all* the animals. These results show a high negative correlation between the bactericidal activity of the serum of these vaccinated animals and the degree of virulence of the organism. They also show that, even in serums from which agglutinins have disappeared, other antibodies for the same organism may be present. Perhaps these latter have more significance in the resistance of an animal than do the agglutinating antibodies.

Within the period in which these tests were made (i. e., 6 to 18 months after vaccination) there was no definite indication of a lowering of the bactericidal titer. That is, about an equal number of serums showed no change in titer as compared with the number which showed a possible reduction in activity. It is not implied, however, that the bactericidal titer of the serum of any of these would remain fixed.

Parallel studies to those reported in this paper are in progress at present on the serums of animals vaccinated as calves with strain 19, having as one objective to test the duration of the immunity induced by vaccination. The serums of these thirty-odd animals have likewise exhibited a differential activity toward strain 19 and a virulent strain of *Br. abortus*. The details of these studies will be reported later.

SUMMARY

The bactericidal action of the serum from both normal and vaccinated cattle has been shown to depend on the combined activity of antibody and complement.

The serum of normal animals usually has an appreciable bactericidal activity at dilutions of 1:40 or 1:80, that of some individuals at 1:160, rarely higher. The serum of animals vaccinated as adults, or nearly so, showed a definite antibacterial activity to *Brucella abortus* at dilutions of 1:1,280 and even at 1:10,240 in some individuals. The serum of these individuals showed partial agglutinating reactions no higher than 1:100, but with that from one cow at 1:200.

In the majority of the tests, undiluted or diluted serum destroyed more organisms of strain 19 than of the more virulent strain.

These tests show that even in the almost complete absence of agglutinating antibodies the serum of a vaccinated animal may exert antibacterial activity against *Br. abortus* greater than that of the serum of normal animals. These results substantiate a previous report on a parallel finding in the serum of animals once infected, but with a titer of agglutinating antibodies no higher than in normal cattle.

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